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and Veterinary Medicine of Bucharest
Faculty of Horticulture

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EFFECT OF FERTILIZATION ON THE GROWTH, NUTRITIONAL AND PHYSIOLOGICAL STATUS OF APPLE PLANTING MATERIAL (*MALUS DOMESTICA* BORKH.) GROWN IN CONTAINERS

Vanya AKOVA, Irina STANEVA, Lilyana NACHEVA, Nataliya DIMITROVA, Angel DIMITROV, Plamen IVANOV, Stefan GANDEV

Agricultural Academy, Fruit Growing Institute, 12 Ostromila, Plovdiv, Bulgaria

Corresponding author email: vannnia85@abv.bg

Abstract

The study was conducted at the Fruit Growing Institute in Plovdiv to establish the influence of different fertilizer rates on the growth, nutritional and physiological status of apple plants of the Florina cultivar, grafted on M9 rootstock, grown in containers (10 l). Variants of the experiment are Variant I - Control (not fertilized), Variant II - $N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, Variant III - $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$ and Variant IV - $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$. In fertilized variants, plants with a height of 144.11 to 152.14 cm and a stem diameter of 9.63 to 10.09 mm were obtained. The plants of the control variant reached average values for height of 92.38 cm and 6.95 mm for stem diameter. The plants of low fertilizer rate were distinguished by the largest volume of the root system (99.44 cm^3). There is a pronounced tendency for a direct increase in nitrogen with increasing fertilizer rate. The experimental data obtained showed that the low fertilizer rate ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$) was optimal and led to the production of high-quality apple planting material suitable for establishing fruit orchards.

Key words*: apple, containers, fertilization, planting material.*

INTRODUCTION

The production of the necessary quantities of planting material is a basic prerequisite for the development of fruit growing. Quality planting material must be produced as it determines the future development of fruit trees.

One intriguing new approach in the production of fruit planting material is container production. This is a relatively new method of producing fruit plants and has many advantages (Haris & Gilman, 1993; Gilman & Beeson, 1996; Mathers et al., 2007). In this way, different types of fruit crops can be successfully grown.

This technology has the following two advantages: it allows almost 100% interception when planting in a permanent place, as planting does not cause stress to the newly planted plants and allows establishing orchards throughout the year (Akova, 2021).

Fertilization is a major agrotechnical practice, and is mandatory for the container growing (Akova et al., 2019; 2020a; 2020b).

Apple is a traditional fruit crop in Bulgaria. Florina is the most common apple cultivar in our country.

In Bulgarian fruit growing, conventional tree production in a nursery is mainly applied, which creates the need to explore the possibilities for producing apple planting material in containers.

The study aimed to determine the influence of fertilization on the growth, nutritional, and physiological status of apple planting material of the Florina cultivar produced in containers.

MATERIALS AND METHODS

In the period 2019-2021, a trial was held at the Fruit Growing Institute in Plovdiv to determine the influence of different fertilizer rates on the growth behavior, nutritional and physiological status of apple plants of the Florina cultivar grafted on M9 rootstock grown in containers.

The fertilizer experiment was set in four variants with ten replications, each plant being a separate replication.

The following variants were tested: Variant I - Control (not fertilized), Variant II - $N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, Variant III - $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$, and Variant IV - $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$. The fertilization was applied several times with increasing rates of nutrients

with the combined fertilizer Kristalon N (20%) - P_2O_5 (5%) - K_2O (10%) - MgO (2%). Optimal soil moisture was maintained in the containers, consistent with the requirements of the crop grown.

The following biometric indicators were reported: stem diameter (mm), plant height (cm), leaf area (mm^2), the volume of the root system (cm^3), and fresh mass of leaves, stems, and roots (g). Chemical analyses of leaf samples for the content of leaf pigments and nutrients were carried out, and the content of nitrogen, phosphorus, potassium, calcium, and magnesium was determined.

The leaf area was measured by scanning the leaves and analyzing the resulting images with specialized software (Gao et al., 2011). The volume of the root system was measured using Burdett's method (1979). The chlorophyll content (*a*, *b*, *a*+*b*) was determined spectrophotometrically in a 95% ethyl alcohol extract.

Table 1. Definitions of measured and calculated chlorophyll fluorescence parameters used in the experiment, based on Strasser and Strasser (1995) and Goltsev (2016)

Chlorophyll Fluorescence Parameters	Description
Measured parameters and basic JIP-test parameters derived from the OJIP transient	
$F_O \sim F20\mu s$	Minimum fluorescence, when all PSII reaction centres (RCs) are open; Fluorescence intensity at 20 µs
F_J	Fluorescence at the J-step (2 ms) of the O-J-I-P transient
F_I	Fluorescence at the I-step (30 ms) of the O-J-I-P transient
$F_M = F_P$	Maximum fluorescence at the P-step when all RCs are closed
$V_J = (F_J - F_O)/(F_M - F_O)$	Relative variable fluorescence at the J-step
$F_V = F_M - F_O$	Variable fluorescence
Quantum yields and probabilities	
F_V/F_M	Maximum quantum efficiency of PS II photochemistry
$\psi_{EO} = 1 - V_J$	Probability (at t=0) that a trapped exciton moves an electron into the electron transport chain beyond QA^-
$\varphi_{EO} = (1- F_J/F_M)$	Quantum yield (at t=0) for electron transport from QA^- to plastoquinone
$\delta_{RO} = (1 - V_I)/(1 - V_J)$	Efficiency/probability (at t = 0) with which an electron from the intersystem carriers moves to reduce end electron acceptors at the PSI acceptor side
Performance indexes	
PI_{ABS}	Performance index of PSII based on absorption
$PI_{total} = PI_{ABS} \times \delta Ro/(1 - \delta Ro)$	Performance index of electron flux to the final PSI electron acceptors, i.e., of both PSII and PSI

Analysis of chlorophyll fluorescence was performed on the youngest fully developed leaves from the top of three representative plants of each variant. The main parameters of the rapid fluorescence of *chlorophyll a* were measured with a portable Handy PEA fluorimeter (Hansatech Instruments, UK). The analyzed sections of the leaves were darkened for 40 minutes with special clips. The induction curves of rapid chlorophyll fluorescence (OJIP test) were recorded for 1 s after illumination with a light pulse (3000 µmol m-2 s-1 PPFD).

Primary data processing was carried out using a PEA Plus program (V1.10, Hansatech Instruments Ltd., UK).

Measured and calculated parameters using this OJIP test (Table 1) were interpreted and normalized according to Strasser and Strasser (1995) and Goltsev (2016).

The results obtained were processed statistically using the method developed by David B. Duncan (Duncan, 1955; Harter, 1960). Software "R3.1.3", in combination with "RStudio-0.98" and installed package "agricolae 1.2-2" (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

The mineral nutrition performed affects the growth manifestations of apple plants (Table 2). All fertilized variants (Var. II, Var. III, and Var. IV) had higher values at all measured characteristics compared to the control (Var. I). The differences were statistically proven.

Table 2. Effect of fertilization on the growth characteristics of apple planting material grown in containers

Variants	Stem diameter (mm)	Plant height (cm)	Root system volume (cm^3)	Leaf area (mm^2)
I	6.95 b	92.38 b	46.67 b	13,685.76 b
II	10.09 a	152.14 a	99.44 a	257,249.52 a
III	9.76 a	148.96 a	66.11 ab	190,959.12 a
IV	9.63 a	144.11 a	55.55 b	155,461.68 ab

The results obtained show that there were no significant differences in the main growth indicators between the fertilized variants (Var. II, Var. III, and Var. IV). The nourished plants (Var. II, Var. III, and Var. IV) had a height of 144.11 to 152.14 cm, and those of the control were characterized by lower average height values (92.38 cm) (Table 2, Figure 1).

The mean stem diameter in control plants was 6.95 mm. Fertilized plants (Var. II, Var. III, and Var. IV) had higher stem diameter values - from 9.63 to 10.09 mm. The differences to the non-fertilized control were statistically proven.



Figure 1. Growth habits of the experimental plants in the different variants at the end of the vegetation



Figure 2. Root system in the different variants

The tree root system volume was measured because, according to Jacobs and Seifert, 2004, this method does not lead to its violation and is relatively fast. Fertilization affects both the above-ground part of the plants and the root system (Akova et al., 2019, 2020a, 2020b, 2022). A significant increase was observed in the root system volume was observed (Table 2, Figure 2). The plants of the fertilizer variants (Var. II, Var. III, and Var. IV) had values from 55.55 to 99.44 cm^2, and those of the control variant were characterized by lower average values (46.67 cm^2). There were proven differences between the variants tested. The fertilizer rate is one of the factors that affects the quality of planting material. Excessively high fertilizer rates can cause growth depression. Variant II (99.44 cm^3) had the highest value for the volume of the root system. The medium and high rates (Var. III and Var. IV) inhibit root growth. The values for the volume of the root system in fertilized plants with the high rate were comparable with those in unnourished plants. A number of authors emphasize a positive correlation between root system volume and subsequent plant development under field conditions, with plants having larger root system volume showing higher survival (Rose et al., 1991a; 1991b; 1992; 1997; Jacobs et al., 2005).

A significant increase in leaf area has also been reported. The plants of the fertilized variants (Variant II, Variant III, and Variant IV) had leaf area ranging from 155461.68 to 257249.52 mm^2, and those of the control variant were characterized by lower mean values (13685.76 mm^2). The high fertilization dose (Variant IV) increased leaf area, but compared to the low and medium doses (Variant II and Variant III), the effect of fertilization was weaker.

The influence of different fertilizer rates on the content of the essential nutrients in the leaves is presented in Table 3.

Table 3. Content of essential nutrients in the leaves

Variants	N (%)	K (%)	P (%)	Ca (%)	Mg (%)
I	1.77	2.29	0.25	2.28	0.72
II	2.27	2.24	0.30	1.29	0.55
III	2.33	2.25	0.31	1.35	0.50
IV	2.35	2.35	0.33	1.23	0.50

During the 2019-2021 study, the applied fertilization had a beneficial effect on the macronutrient content in the leaves of the observed fruit species.

There was a pronounced tendency for a directly proportional increase in nitrogen with increasing fertilizer rate. In control plants, the nitrogen content was in the low supply range. The N content of fertilized plants was in the range of 2.27-2.35%, which corresponded to adequate supply, and was about 50% higher than that of the control plants (1.77%). The K content was in a range of high reserve, with the values of this nutrient being optimal in all variants.

The results regarding the P content showed a clear trend of increase with increasing fertilizer rate, and in all variants, the values for this nutrient were optimal.

The values for Mg content in leaves were higher in the control plants. The same trend

was observed with regard to the concentration of *Ca*. This was probably due to the antagonism between the elements potassium and calcium. The application of increasing fertilizer rates of the element potassium leads to a lower absorbtion of *Ca* from plants. Despite this, the values for *Ca* in the leaves of fertilized plants were within optimal limits.

Table 4. Effect of fertilization on the chlorophyll content in the leaves

Variants	Chl. *a* mg/g (DW)	Chl. *b* mg/g (DW)	Chl. *a+b* mg/g (DW)	Carotenoides mg/g (DW)
I	1.50 b	0.54 b	2.05 b	0.54 b
II	3.54 a	1.10 a	4.64 a	1.18 a
III	4.17 a	1.40 a	5.57 a	1.49 a
IV	4.15 a	1.27 a	5.42 a	1.55 a

It has been found that the introduction of increasing concentrations of K and P leads to an increase in the rate of photosynthesis but does not significantly affect the chlorophyll content of the leaves (Wang et al., 2022). We observe such a trend in our study. From the experimental data obtained, it can be seen that statistically proven differences between the individual fertilization variants have not been found (Table 4). A higher content of *chlorophyll a* distinguishes plants of fertilizer variants. The *chlorophyll b* content was lower than that obtained for *chlorophyll a*, but a positive effect of feeding was observed in all fertilized variants. The results in the total *chlorophyll a+b* showed an increase in the values of the fertilization variants, suggesting a good adaptation of the plants to the growing conditions. The highest values for *chlorophyll a+b* were recorded when the medium fertilizer rate was applied (var. III) ($N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$). With an increase in the fertilizer norm, there was a gradual increase in the content of carotenoids. From the results obtained, we can conclude that the applied fertilization favors the accumulation and maintenance of chlorophyll in the leaves during the growing season, which leads to an improvement of the photosynthetic activity of the plants and their good adaptability to the growing conditions.

Although the content of photosynthetic pigments is not the only indicator of plant photosynthesis, their increase can be considered as an expression of a better structuring of the photosynthetic apparatus under conditions of improved nutrition.

Another indicator of the functional activity of plants' photosynthetic apparatus is chlorophyll fluorescence. The analysis of rapid fluorescence induction curves of *chlorophyll a* (OJIP test) links the structure and functionality of the photosynthetic apparatus. It allows a rapid assessment of plant viability, especially under stress (Strasser et al., 2000; 2004).

Table 5. Effect of fertilization on the biomass of leaves, stems, and roots of plants

Variants	FW		
	Leaves (g)	Stems (g)	Roots (g)
I	11.52 b	60.67 c	32.33 b
II	108.13 a	135.77 a	236.13 a
III	110.31 a	99.67 b	212.13 a
IV	98.76 a	89.52 b	206.58 a

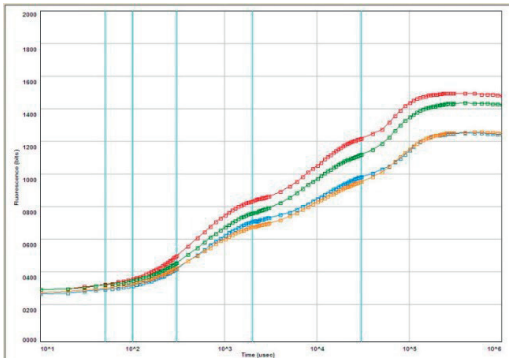


Figure 3. Induction curves of rapid chlorophyll fluorescence (OJIP test); (----) Control without fertilization; (----) variant II; (----) variant III; (----) variant IV

Table 6. Basic chlorophyll fluorescence parameters (JIP test) of apples grown in containers at different levels of fertilization

Variants/ Parameters	I (Control)	II	III	IV
F_0	294 a	305 a	328 a	319 a
Fm	1264 c	1273 c	1368 b	1447 a
Fv	970 c	969 c	1040 b	1128 a
Fv/Fm	0.763 b	0.790 a	0.750 b	0.775 a
ψE_0	0.546 a	0.599 a	0.492 a	0.585 a
φE_0	0.417 a	0.456 a	0.374 a	0.454 a
δR_0	0.484 a	0.519 a	0.493 a	0.457 a
PI abs	2.69 b	3.63 a	2.26 c	3.73 a
PI total	2.87 c	4.04 a	1.77 d	3.59 b

In the control and the three fertilizer variants, the rapid chlorophyll fluorescence curves had a

typical F_0 to F_m level shape with pronounced J and I phases (Figure 3), indicating that the apple plants included in the experiment were photosynthetically active (Yusuf et al., 2010).

No significant differences were reported in the values of the minimum fluorescence (F_0) of control and treated plants (Table 6.). The highest value of the maximum fluorescence (F_m) was measured in variant IV, and the lowest was in the control. Despite fluctuations in initial (F_0), maximum (F_m), and variable (F_v) fluorescence, the quantum yield (F_v/F_m) reflecting the potential photochemical activity of photosystem II (PS II) ranges from 0.750-0.790 and corresponds to normal (0.750-0.830) in healthy, unstressed leaves (Bolhar-Nordenkampf & Oquist, 1993). This shows that a normally developed photosynthetic apparatus was functioning in all the studied variants. No statistically significant differences were reported for three important parameters of the JIP test – ψE_0, φE_0, and δR_0 in plants grown with and without fertilization. The Total Performance Index (PI total) reflects the functional activity of PS II and PS I and the electron transport chain between them. PI total is closely related to total plant growth and survival under stress and is considered a very sensitive indicator of the JIP test. The highest PI total was reported in variant II (4.04), which was significantly higher than in the control (2.87). The highest PItotal of the plants fertilized with the low dose (variant II) corresponded with their larger biomass, height, stem diameter, and leaf area and clearly showed the effectiveness of the applied treatment (Tables 2, 5, 6). Improved plant nutrition contributes to more efficient development and structuring of the photosynthetic apparatus, which in turn is a prerequisite for more intense photoassimilation and biomass accumulation. The lower values of some of the main chlorophyll fluorescence parameters (F_v/F_m, φE_0, ψE_0, PI abs, PI total) measured on the leaves of variant III are formally in contradiction with the more intensive plant growth of this variant compared to the control. This could be related to the growth rate of these plants - by the the time of measurement at the end of the vegetation, the shoots of the contro plants stopped their growth and formed an apical bud. The plants of variant III continued growing in height, and their top leaves (on which the measurement is made) were physiologically younger than those of the control ones.

CONCLUSIONS

All three fertilizer rates ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$, and $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$) significantly affect stem diameter and plant height growth resulting in apple planting material with larger sizes than the control (non-fertilized) trees.

The high fertilization rate ($N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$) inhibits root growth, and the values for the root system volume are commensurate with those of unnourished plants.

The fertilizer rate is one of the factors on which the quality of planting material depends. The low fertilization rate ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$) is optimal. It stimulates the growth and biomass accumulation, has a beneficial effect on the content of the essential nutrients and chlorophyll in the leaves, and leads to the production of high-quality apple planting material suitable for planting in orchards.

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THE HISTORY OF PALM CULTIVATION IN IRAQ

Imad Jabbar Wadi AL-SUWAID, Ana Cornelia BUTCARU, Cosmin MIHAI, Florin STĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: emadjabbar74@yahoo.com

Abstract

The date plant with the scientific name of Phoenix dactylifera L. has a history of cultivation of more than 5000 *years. The date palm tree is used for various purposes such as food, shelter, fibers and fuel. Dates are one of the top 5 products produced in the world and after wheat, tomatoes and barley it is the fourth product produced in the world. Arab countries have more than 70% of date palm trees. More than* 67% *of dates are produced in Arab countries. The cultivation of dates in Iraq has a long history. Iraq has been one of the top ten date producing countries for a long time (the last two decades). Dates are Iraq's second export product. However, various factors such as war, sanctions, environmental tensions, and pests and diseases reduced the production of dates and destroyed a wide area of date orchards. Fortunately, nowadays, according to various management programs for the production and export of this product, as well as various research conducted with the aim of increasing the productivity and quality of the fruit, date production in Iraq has been progressing. So, in* 2022*, Iraq was the first exporter of dates in the world.*

Key words*: export, fertilization, pollination, production.*

INTRODUCTION

The date palm plant with the scientific name *Phoenix dactylifera* L. has a history of cultivation of more than 5000 years (Zohary & Hopf, 2000; Zaid & Arias-Jimenez, 2002; Al Farsi & Lee, 2008). The word "phoenix" is probably derived from the Greek word for the red or purple fruit, and the “dactylifera” from the Greek daktulos, which means finger like (Zabar and Borowy., 2012). This species has economic, symbolic and cultural importance in all cultivated areas such as Morocco, Arabian Peninsula and India (Fuller and Stevens, 2019; Tengberg, 2012). The palm tree is used for various purposes such as providing food, shelter, fibers and fuel (Plotkin & Balick, 1984; Mallaki & Fateh, 2014). Palm has been repeated about 64 times in the holy books of Torah, the gospel and the Qur'an. Due to the fact that the palm is in the group of evergreen plants, the ancient Egyptians considered them a symbol of immortality. The palm tree is a symbol of peace, victory and fertility in many cultures and has a special place in ancient Egypt (Farmhand, 2019). The date palms are cultivated due to the production of carbohydrate-rich fruits in tropical and arid areas where plants have little ability to grow (Shrinath Baliga et al., 2011). In addition to being consumed as food, the sweet date fruit has long played a role as an important product in agricultural livelihood and economic prosperity (Fuller and Stevens, 2019; Tengberg, 2012). The total number of date palm trees in the world is estimated at 120 million trees. Arab countries have more than 70% of date palm trees. Therefore, 67% of the world's date production is in Arab countries (El-Juhany, 2010). It should be mentioned that the date palm also has ornamental importance (Abdel Fattaah et al., 2017). According to the FAO statistics in 2022, the total production of dates in the world and Iraq was 9,747,570 and 715,293 tons, respectively (FAOSTAT, 2024).

ORIGIN AND DISTRIBUTION

The date plant has been cultivated since ancient times (Riad 2006). The dates appear to be one of the few plant species that have been domesticated in their native desert environment (Zohary and Hopf, 2000). Dates were cultivated in North Africa and Central Asia nearly 1,500 years ago (Zabar and Borowy, 2012). It is probable that the Middle East was a region for the date to become native in the fourth period

before Christ. It was probably later transferred to North Africa during the Roman period (Munier, 1973; Nixon, 1951; Tengberg, 2012). In Christianity, the leaves of the date palm tree are used during the Easter celebrations (Zohary and Hopf, 2000). In Islam, the date palm tree is mentioned repeatedly in the Qur'an and has a special importance in religion and Islam (Zabar and Borowy, 2012). The date palm tree is depicted in the ancient Babylonian and Assyrian tablets. In addition, this tree is also mentioned in the Palestinian, Libyan, Syrian and Egyptian writings (Zabar and Borowy, 2012). Recent studies have shown that *P. dactylifera* was not the result of hybridization between two wild *Phoenix* species. Based on genetic studies, it has been reported that *P. dactylifera* was probably domesticated from the same wild population (Pintaud et al., 2010). In confirmation of this hypothesis, another study found that *P. dactylifera* grown in the enclosed area of the Hajar Mountains, Oman, was wild (Gros-Balthazard et al., 2017). Ancient evidence of wild date palms has been found in the ancient remains of West Asia. In the Shanidar cave in Iraq, more than 50 kg of pollen and ca. 46 kg phytoliths associated with date palms have been observed (Henry et al., 2011; Solecki and Leroi-Gourhan, 1961). Date palms Phytoliths found in Iraq have been from sediments dating back to 10,000 BC (Altaweel et al., 2019). From excavations in the Levantine region, a nearly 19,000-year-old stem burned and 69-69 Ka phytoliths were discovered (Henry et al., 2004; Liphschitz and Nadel, 1997). It is thought to have originated in the Mesopotamian region (southern Iraq) or western India (Zabar and Borowy, 2012). Today, the date palms are grown and cultivated in the Middle East, North Africa, parts of southern and central America, southern Europe, India and Pakistan (Zaid & Arias-Jimenez, 2002; Al-Shahib & Marshall, 2003).

BOTANICAL

About 19 species have been identified in the *Phoenix* genus (Zabar and Borowy, 2012). Date palm is a monocotyledonous, perennial, diploid and dioecious plant (2n=2x=36). This plant belongs to *Arecaceae* family (Barrow, 1998). The morphology of male and female date palm inflorescences is different from each other. In both inflorescences, there is a fibrous and hard covering called spots, which protect the flowers from direct sunlight in the early stages of growth (Chao and Krueger, 2007). This plant grows in arid areas. The length of the tree reaches 30 to 36 meters. The trunk diameter of date palm is more than 50 cm. The trunk of the plant is covered with upward, woody and resistance leaves. After 6 to 16 years, the offshoots grow around the main trunk (Zabar and Borowy, 2012).

THE IMPORTANCE OF THE DATE PALM

Date fruit is a rich source of nutrients such as antioxidants, minerals, carbohydrates, protein and fiber (Noutfia and Ropelewska, 2023). About 72-88% of date fruit is made up of carbohydrates, which are mainly glucose, sucrose and fructose. In addition to carbohydrates, dietary fiber, a small amount of protein and fat, vitamins such as riboflavin, thiamin, biotin, folic acid and ascorbic acid are present in date fruit. The energy value per 100 grams of date fruit is equal to 314 kcal (Al-Farsi and Lee, 2008). Mesocorp is rich in various minerals such as iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, sulfur, boron, selenium and zinc (Ali Mohamed & Khamis, 2004; Al Farsi & Lee, 2008). In addition, there are abundant amounts of compounds in it, such as: antioxidant, antimutagenic, anthocyanin, phenolic and free and bound acids (Vayalil, 2002; Al-Farsi et al., 2005), 16 types of amino acids, vitamins A, B, B2, dietary fibers and fatty acids (Karasawa et al., 2011; Al-Shahib & Marshall, 2003; Ahmed et al., 1995).

The therapeutic effects of this plant include antioxidant (Kchaou et al., 2013), anti-hot flushing and anti-cancer (Rahmani et al., 2014), antimicrobial (Shariati et al., 2010; Shakiba et al., 2011; Al-daihan & Bhat, 2012; Al-Zoreky & Al-Taher, 2015), antifungal, neuroprotective (Pujari et al., 2014) and hepatoprotective agents (Singab et al., 2015). Dates are traditionally used in the treatment of diabetes and blood pressure (Hifnawy et al., 2016). Spathe extract is used as a flavoring in drinks (Al-Taher, 2008a; Demricia et al., 2013). Phytochemical analysis

of Spathe has shown that Spathe contains various compounds such as sugar, furfural, calcium pectate, protein, wood ash, three types of coumarin, 1 and 0 Dimethoxyls, 1 and 4 dimethylbenzene and plant sterols (Demrici et al., 2013).

PROPAGATION AND POLLINATION

Dates are propagated by seeds, vegetative propagation (offshoots) and tissue culture (Zabar and Borowy, 2012). Seed propagation is mainly not done due to the production of different plants and the high probability of decreasing fruit quality (Moustafa et al., 2010). Date palm vegetative propagation is accomplished by offshoots grown in the mother plant (Alihouri and Dialami, 2010; Tengberg, 2012). This method of propagation in the palm is very old. The evidence suggests that the propagation of the date palm by this method is related to the second half of the third millennium BC (Tengberg, 2012). Tissue culture technique is one of the high-speed propagation methods in plants. Shoot tips, embryo culture, and bud culture are commonly used as the explants in this technique. It should be mentioned that leaves, inflorescences, stems and roots are also used as the explants (Al-Sakran and Muneer, 2006). Pollination of female flowers in date palm is one of the most important stages of plant growth (Janick, 2005; Moustafa et al., 2010). The ideal temperature for pollen seed germination is 35°C (Zirari, 2010). This process has probably been used in southern Mesopotamia since the late 4th millennium BC (Landsberger, 1967; Tengberg, 2003). It was first mentioned in the texts in the 18th century BC by the Babylonians. After that, it was also seen in the Assyrian iconography (Sarton, 1934). Dates flowering occurs at a temperature of 18°C and fruit formation occurs at a temperature higher than 25°C (Zaid and Wet, 2002a). The number of 4-10 male trees is sufficient to pollinate 10 female trees (IQBAL et al., 2004). Pollination is done during the flowering season (February and March). Pollination is done manually by placing two or three pollen-carrying filaments in the female flower cluster. Mainly, due to the high cost of the worker specializing in pollination, farmers allow pollination to be done with the wind, which affects the fruit productivity and quality (FAO and AOAD, 2023). Date pollination is also possible through insects (Nixon and Carpenter, 1978; Zaid and Wet, 2002c). In general, there are few male trees in the date palm garden, whose pollen is used for manual pollination. The pollination of 60-80% of female date flowers produces a sufficient and appropriate amount of fruit (Nixon and Carpenter, 1978; Zaid and Wet, 2002c). Different types of pollen, affect the size and shape of seeds (Chao and Krueger, 2007). The Iraqi date male cultivars that are used to produce pollen, include Ghanami Ahmer, Ghanami Ahmer, Khakri Kratley, Khakri Smasmi, Khakri Wardi and Khakri Adi. The difference in these cultivars is determined by the size of the pollen grains. The largest pollen grain size was observed in Khakri Kratley and the smallest pollen grain was observed in Ghanami Ahmer. Ghanami Ahmer has better quality in bunches and pollen grains in each cluster compared to other cultivars. The use of pollen from Resasi in the rootstock of Khadrawy material increased the maturation period. However, no difference was observed in Maktoom fruits. The use of Ghanami Ahmer pollen in Halawy trees increased the percentage of maturity. However, it had no effect on Sukar cultivar (Ibrahim, 2008). In a study conducted in Iraq, which examined the pollen grains and the plant growth regulator in Sultani cultivar, it was found that the use of 50 $mg.l^{-1}$ NAA along with Ghannamiahmer cultivar had a positive effect on the fruit formation. However, compared to the other pollinator cultivars (Ghannamikhder and Ghannamiahmer), Khukri cultivar increased the fruit size and weight. In addition, it significantly improved the content of fruit dry matter and total sugar compared to other cultivars. Also, the use of Ghannamiakhder significantly increased the bunch weight in the rutab stage (Aubied et al., 2019).

APPLICATION OF DATE PALM TREE

Date palms are used in the production of paper (Jonoobi et al., 2019). In addition, date palm is also effective in absorbing heavy and toxic elements. Dates Palm can be used in the absorption of lead toxic element (Muthusaravanan et al., 2018; Janani et al.,

2019; Alghamdi, 2016). The fibers and petioles of dates in aqueous solution have the potential to absorb lead (Al-Haidary et al., 2011). It should be mentioned that various factors such as PH of the solution, absorption dose, duration of exposure in the solution, ionic strength and temperature are effective on the amount of silver ion absorption. In general, the use of date palm waste in the absorption of lead ions is an efficient and low-cost method (Jonoobi et al., 2019). Another use of date palm is the production of Wood Composites (Jonoobi et al., 2019; Zadeh et al., 2017; Gheith et al., 2019). Among the various applications of date palm in composite production, Date palm propylene composite, Propylene reinforced with date palm fibers, Date palm fiber/polyester composite, Reinforced ethylene terephthalate composite, Starch-based biodegradable composite, Natural mortar reinforced with date palm, Wood-polymer composite with date palm and epoxy matrix, OSB from date palm, Date palm MDF, Particleboard from date palm, Wood-cement composite with date palm and Gypsum board from date palm (insulation board) can be mentioned (Jonoobi et al., 2019). The use of palm fibers has been effective in strengthening polypropylene and low-density polyethylene (Alzebdeh et al., 2016). Another use of date palm is the production of biochar and its use as a source of fuel and energy (Jonoobi et al., 2019).

PALM CULTIVATION IN IRAQ

Dates are Iraq's second largest export crop after petroleum. As a result of the Iran-Iraq war, the main center of date palm cultivation in Iraq (Basra suburb) lost a large number of date palm trees and became a date tree desert. During the war in 2003, the date palm production industry suffered seriously. Today, several programs have been implemented by the Iraqi authorities to revive date palms (Khierallah et al., 2015). It has been said that the earliest cultivation of dates was done in Eridu, around 4000 B.C. Today it is known as Tell Abu Shahrain (Khierallah et al., 2015). The average economic life of the palm garden is about 40 to 50 years. Some of them have reproductive capacity for more than 150 years (Nixon and Carpenter, 1978). The war and various sanctions imposed on Iraq had a negative effect on crop production and genetic diversity (Khierallah et al., 2011). Understanding the amount and distribution of genetic diversity in the genetic pools plays a decisive role in breeding programs and genetic conservation (Jubrael et al., 2005). The use of morphological traits is used to describe genetic diversity in date cultivars (Barreveld, 1993). In a study conducted by Khieralla et al. (2011) in Iraq, it was found that the genetic diversity in 30 date palms (24 female and 6 male cultivars) by 22 microsatellite primers showed high polymorphism. The heterozygous rate for all cultivars was 0.503. The genetic distance between the cultivars varied from 0.171 to 0.938, which indicates diverse relationships. The results showed that the Ghanami Akhder cultivar was very different from the Ghnami Ahmer cultivar. While, two cultivars of Jamal Al-Dean and Qitaz were closely related to each other.

PRODUCTION OF DATES IN THE WORLD AND IRAQ

According to the data of FAO last 20 years (1999-2022), dates are one of the top 5 products produced in the world, and after wheat, tomatoes and barley are the fourth produced in the world. In 2022, date production came in the second place after wheat. This clearly shows the importance of this product. An investigation on FAO data (FAOSTAT, 2024) showed that from 1999 to 2022, more than half of dates are produced in Asia (59.9%) and then in Europe (39.9%) (Figure 1a). In the last 20 years, Egypt has produced the largest amount of dates (1,369,385 tons). In this ranking, Iraq is ranked fifth after Egypt, Saudi Arabia, Iran and Algeria. The amount of date production in Iraq in this period was 647,480 tons (Figure 1b).

The study of date production in Iraq showed that in 2022, Iraq will rank sixth after Egypt, Saudi Arabia, Algeria, Iran and Pakistan (Figure 2). In 2022, the total amount of dates produced in Iraq was 715,293 tons and the harvest area was 278,159 hectares.

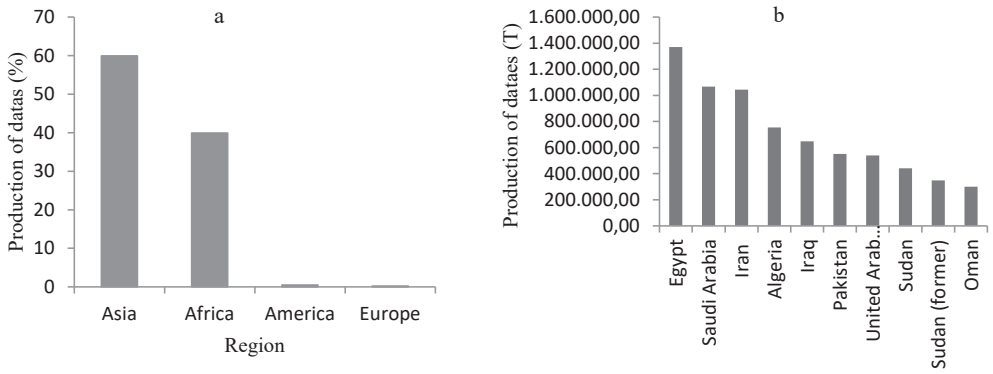


Figure 1. Production of dates by region (a) and top ten producers (b) from 1999 to 2022 (Source: FAOSTAT, March 14, 2024)

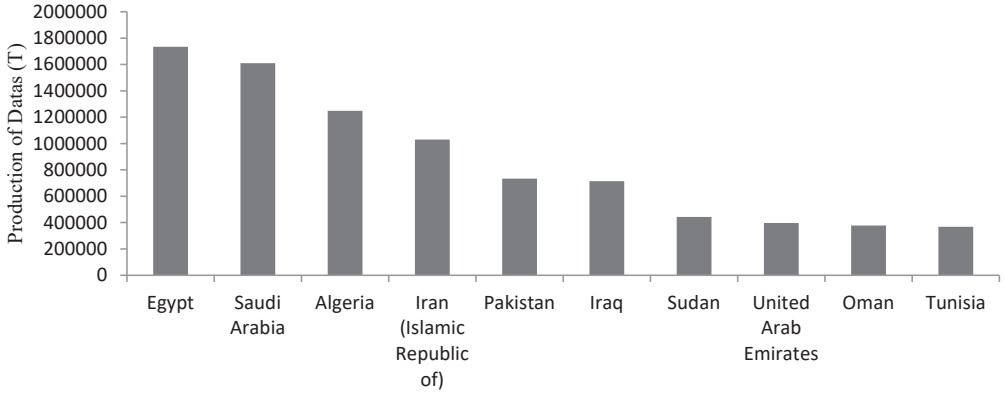


Figure 2. Top ten producers of dates in 2022 (Source: FAOSTAT, March 14, 2024)

EXPORTING AND IMPORTING DATES IN THE WORLD AND IRAQ

The data related to the last 20 years show that Iraq was the second largest exporter of dates in the world after the United Arab Emirates (193,690 tons) with the export of 171,925 tons of dates.

Iran, Pakistan and Saudi Arabia are in the next ranks with 151,766, 109,370 and 97,126 tons respectively (FAOSTAT, 2024).

In contrast, five countries include India (289,800 tons), the United Arab Emirates (109,957 tons), Morocco (53,019 tons), Yemen (30,913 tons) and France (30,760 tons) are the five importers of dates countries in the last 20 years.

In 2022, the amount of dates exported from Iraq was 274,179 tons and the amount of imported dates was 3469 tons.

In addition, in this year, Iraq was the first exporter of dates and India was the first importer (Figure 3).

Table 1 shows the import and export of dates in Iraq from 1999 to 2022.

The import of dates in Iraq started in 2014 (Table 1). According to FAO data, the price per ton of dates in Iraq in 2022 was 826 dollars.

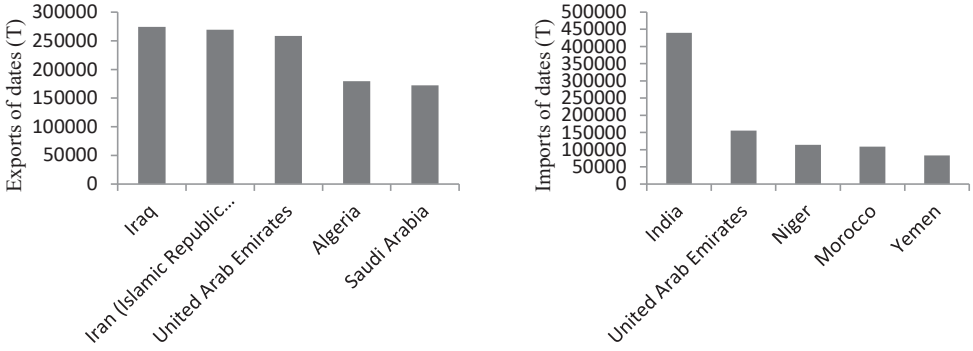


Figure 3. Top five export (a) and imports (b) of dates in 2022

Table 1. Exports and imports of Dates in Iraq from 1999 to 2022 (FAOSTAT, 2024)

Year	Exports (ton)	Imports (ton)
1999	30000	-
2000	30000	-
2001	4000	-
2002	8000	-
2003	5016	-
2004	23485	-
2005	149660	-
2006	42776	-
2007	221016	-
2008	296642	-
2009	198777	-
2010	126731	-
2011	138437	-
2012	177498	-
2013	144607	-
2014	362492	8440
2015	296979	10628
2016	321661	13528
2017	254752.2	13947.33
2018	263465.6	21926
2019	230152.7	14000
2020	208151.5	6162.28
2021	317736.9	2899
2022	274180	3469.57

CULTIVARS CULTIVATED IN IRAQ

More than 400 different cultivars of dates are cultivated in Iraq. The difference between them is determined based on the appearance and texture of the fruit. Accordingly, dates are divided into three types: soft, semi-dry and dry types. This division is based on the texture or consistency of the fruit at the time of its ripening. The amount of moisture in soft, semi-dry and dry dates is more than 30%, between 20-30% and less than 20%, respectively. Khastawi, Barhee, Halawy, Hayany and Khadrawy cultivars belong to the soft date group. Halawi, Dayri and Khadrawy cultivars are classified as semi-dry cultivar and Zahdi and Sayer cultivars are classified in the dry cultivar group. The ripening of date fruit takes place in five phases: Hababouk, Kimri, Khalal, Rutab, and Tamar (Zabar and Borowy, 2012).

FERTILIZATION

Date palm shows a good response to chemical and organic fertilizers. In the modern date cultivation system, the amount of nitrogen used in the plant is 1.0 to 2.5 kg/tree/year. The amount of phosphorus required for the plant is 0.5 kg/tree/year and the amount of potassium required is 1.5 kg/tree/year. Animal fertilizers and chicken fertilizers are also used as organic fertilizers in the plant. Generally, phosphorus, potassium and animal fertilizers are used in November and nitrogen fertilizers are used in January, March and May (FAO and AOAD, 2023). It should be mentioned that the date palm, like other plants, has shown a good response to foliar application of nutrients. The use of urea (N 46%) at a concentration of 1% in Hellawi, Shahany and Khaidrawi cultivars improved yield, leaf nitrogen content, increased pulp weight, fruit length and diameter, fruit quality and reduced fruit drop, and increased dry weight biomass and the percentage of fruit ripening (Abbas et al., 2007; Khayyat et al., 2007; Shareef, 2011a; Shareef, 2011b). In Shany cultivar, spraying the plant in the Khalal stage using Zinc sulfate (300 and 600 ppm) had a significant effect on the pulp weight, fruit length and diameter, increase in total soluble solids, yield and quality of the fruit (Khayyat et al., 2007). The use of Boron in Zehdi and Khestawi Medjool cultivars increased the concentration of B in the fruit, increased the seed weight, fruit flesh, cluster weight and total yield, bunch weight, fruit set, fruit quality and fruit physical characteristics (Al-Dulaimy and Al-Kraidi, 2017; Al-Hajjaj and Ayad, 2018). Also spraying the plant with calcium in Behri and Sayer cultivars (Jasim et al., 2016), phosphorus in Shukar cultivar (Fasal et al., 2014) and sodium selenite in Zaghloul cultivar (Gad El-Kareem et al., 2014) were effective in improving the plant growth and performance indicators. It is noteworthy that the positive effect of foliar spraying in date palm by bio-stimulants has been reported in several studies (Shareef, 2020; Shareef et al., 2017; Attaha and Al-Mubarak., 2014; Taha and Abood, 2018; Al-Wasfy, 2013). In the research conducted in Iraq in Khastawi cultivar, the use of nanofertilizers and NPK improved the traits related to the fruit and the growth (Jubeir and Ahmed, 2019). The study of chemical, organic and biological fertilizers in Khastawi cultivar in Iraq showed that chemical fertilizers have been more successful in improving vegetative traits in the plant compared to other sources of fertilization. The use of humic acid had the greatest effect on leaves' dry matter percentage, the length and width of the leaflet, leaf length and leaf number (Kredi and Al-Ali, 2023). In another research in Zahdi cultivar in Iraq, it was found that the use of urea at the rate of 2 grams recorded the highest indicators related to the yield. On the other hand, the use of urea had no significant effect on the nitrate content in the fruit. In addition, foliar spraying of the plants using seaweed extract was also effective in improving growth indicators related to the fruit (Murad and Al-Dulaimy, 2021).

FRUIT THINNING

Fruit thinning in the dates is one of the most important steps to improve the quality of the fruit in the plant. Fruit thinning is done manually, mechanically or chemically. Fruit size is directly affected by thinning (Dennis, 2000; Link, 2000). Thinning at least 30% of the bunch per date palm and the fruit thinning by removing 30% strand tips or removing 30% of the total number of strands from the center of each bunch significantly increases other fruits,

total soluble solids and fruit sugar content (Al-Wasfy and El-Khawaga, 2008). In Kur cultivar, thinning of 50% strands recorded the highest fruit size and improved fruit indicators related to fruit taste and flavor (Soliman, 2012). In Arab countries, this is done in two methods: 1) cutting and removing the weak bunches after ensuring successful pollination and 2) cutting and removing strands. In the second method, about one-third of the upper part of the flowering strands are removed during pollination. About one-third of half of the fruit-bearing strands after successful pollination are also removed during the fruit formation stage. This process increases fruit quality and reduces the fruit bunch weight (FAO and AOAD, 2023).

PESTS AND DISEASES

In Iraq, a set of different factors such as biological and non-biological parameters are effective in reducing the growth and productivity of dates. One of the most important pests that damage date palm trees in Iraq are Old World date mite, borers, dubas bugs and lesser date moth. Rotting diseases are another limiting threat to date palm cultivation in Iraq (Al-Jboory, 1999; 2001). One of the important pests that involved the date palm is Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier) (Vidyasagar and Aldosari, 2011). In 2000, according to the National Date-Palm Breeding and Improvement Program in Iraq, it was approved and tried to implement correct Mediterranean programs in all stages of planting, harvesting and catching. At first, this program achieved significant success, but with the political and administrative changes in 2003, there was a gradual decline in the production and productivity of dates in Iraq. Studies related to the date pests began in 1912, at the same time as the presence of aphids. Important date palm pests in Iraq include Longhorn date palm stem borer (*Jebusaea hammerschmidti* Reich), Fruit stalk borer (*Oryctes elegans* Prell), Frond borer (*Phonapate frontalis* Fahraeus), Dubas bug (Old World date bug) (*Ommatissus lybicus* de Berg.), Lesser date moth (*Batrachedra amydraula* Meyrick), Greater date moth (*Arenipses sabella* Hampson), Termite (*Microcerotermes diversus* Silvestri), Parlatoria date scale (*Parlatoria blanchardii* Targioni) and Ghobar mite (Old World date mite) (*Oligonychus afrasiaticus* McGregor) (Khierallah et al., 2015). It has been reported that six different species of borers have attacked dates in Iraq, causing a lot of economic damage. Four of these six species belong to the oryctes species (Khalaf and Al-Taweel, 2014). Inflorescence rot (*Mauginiella scaettae*), Black scorch (fool's disease) (*Thielaviopsis paradoxa*, *Chalaropsis radiciala*), Diplodia disease (*Diplodia phoenicum*), Graphiola leaf spot (*Graphiola phoenicis*), Belaat disease (*Phytophthora* sp.) and Brown leaf spot (Alternaria spp.) are one of the most important pathogens of dates in Iraq. Among the important diseases of date palm in Iraq, neck bending, dry rot and growth stunting, frond malformation and dwarfing, and heart wet rot can be mentioned. These diseases are caused by different fungi (Khierallah et al., 2015).

IRRIGATION

The date palm has a minimal water requirement and has a very high tolerance to harsh climates and high levels of salinity stress (Taha and Khalifa, 2022; Alhammadi and Kurup, 2012). However, proper irrigation is necessary for the production of high quality and yield crops (Bazza, 2008). Date palm irrigation is mainly done traditionally as floodwater irrigation (Ishfagh, 2002). One of the irrigation management methods is the use of deficit irrigation (Ghazzawy et al., 2022). In the study of the effect of regulated deficit irrigation in southern Iraq, it was found that the use of this irrigation system resulted in saving 5 to 39 percent of irrigation water. The best performance occurred in full irrigation. In general, it was found that the date palm required regular irrigation during its growth; however, the use of a deficit irrigation system (0-75% ETc) can be used in the study regions (Al-Mansor et al., 2021). Al-Baker (1972) reported the amount of water required for a mature palm to be between 115 and 306 m^3. It should be mentioned that the water requirement of date palm is different in each country and even in different regions of each country (Al-Baker, 1972). The total amount of water required annually in southwestern Arabia is 136 m^3 per tree (Al-Ghobari, 2000). It has been explained that the annual water requirement of date palm

varies from 55 to 137 m^3 per tree in the eastern regions and from 78 to 195 m^3 per tree in trickle and surface irrigation in central regions (Alazba, 2004). Adul Naki and Aslam (2005) estimated the total annual water depth required by date palm to be 2700 and 3000 mm. In Mohammad et al. (2021) research, the annual water requirement of date palms in the controlled sub-surface drip system with sensor-based irrigation scheduling method (S-BIS), time-based irrigation scheduling method (T-BIS) and traditional surface irrigation (TSI) were 21, 22.8 and 58.7 m^3 per tree, respectively. It has been reported that the average amounts delivered to date palms were 108, 216 and 324 m^3/tree/year for water regimes of 50%, 100% and 150% of evaporation rate, respectively (Al-Amoud et al., 2000). In the study of Taha and Khalifa (2022), which studied drip irrigation in the Barhi cultivar, it was found that with the decrease of ETo from 120 to 60%, the yield of the plant decreased by 9.2%. The date palm has the ability to tolerate water shortage in the middle and end of the growing season. Therefore, the use of deficit irrigation is effective in this plant (Molden, 2007). In a research conducted in the Basra region of Iraq, it was determined that the use of underground saline water in the date palm irrigation system is one of the best solutions considering the current drought and temperature conditions. In this study, the use of underground water with a salinity of 10 $dS.m^{-1}$ every four days in the Sayer cultivar improved the growth indicators such as leaf chlorophyll content, plant height, number of new leaves and relative leaf water content (Shareef et al., 2021). Date gardens in Basra are affected by the tides of the Persian Gulf (Zabar and Borowy, 2012). Salinity tolerance is higher in this fruit tree compared to other fruit trees (Alhammadi and Kurup 2012). Date palm can tolerate soil salinity up to 4 $dS.m^{-1}$ without reducing yield. However, water deficit may reduce yield (Taha and Khalifa, 2022).

THREATENING FACTORS OF DATE CULTIVATION IN IRAQ

Various environmental factors such as biotic and abiotic stress (drought and salinity stress) and excessive harvesting are the factors that threaten date cultivation in Iraq (Jaradat and Zaid, 2004). In addition, the reduction and loss of vegetation in some areas due to overgrazing and over-harvesting of wood by individuals causes destructive wind and storms, resulting in soil erosion, reducing the infiltration of rainwater into the soil and increasing the formation of floods and runoff. These factors affect the amount and quality of underground water and soil and ultimately increased the salinity of water and soil. The formation of fine dust and the movement of sand by wind and storm also significantly affect date palm cultivation in Iraq (Amer and Hussain, 2006).

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APPLICATION OF BIOPOLYMERS FOR FUNGAL PATHOGEN CONTROL IN THE STORAGE OF FRUITS

Diyana ALEKSANDROVA[1], Sashka SAVCHOVSKA[1], Gabor ZSIVANOVITS[2]

[1]Agricultural Academy - Sofia, Fruit Growing Institute - Plovdiv, Ostromila 12, Plovdiv, Bulgaria
[2]Agricultural Academy - Sofia, Institute of Food Preservation and Quality, Plovdiv, Bulgaria

Corresponding author email: aleksandrova_diyana@abv.bg

Abstract

The edible coatings of biopolymers such as alginate and chitosan offer promising opportunities for improving the storage of fruits by reducing losses due to fungal diseases. However, it is important to conduct further research to optimize the conditions for their application and ensure their safety and effectiveness in real-world conditions. Nectarine fruits were artificially inoculated with Monilinia spp. and, one day later, coated with 1% chitosan and alginate films. Characterisation of the differently coated fruit was traced during refrigeration. The inhibitory effect of coatings was monitored by measuring the diameter of the lesions on fruits over ten days, and the water loss by measuring milligrams of the fruits. The highest percentage of repression of fungal disease was observed in chitosan coating. The alginate coating has shown a positive effect on fruits during storage to save water contained and extend shelf-life time. The results obtained were compared with the control fruits which were not-application of biopolymer film.

Key words*: Monilinia spp. chitosan, alginate, peach, post-harvest, waste reducing.*

INTRODUCTION

The peach (*Prunus persica* L.) is a preferred fruit by consumers due to its exotic taste and nutrient-rich fruits. Peaches contain several antioxidant compounds, including vitamins, phenols, carotenoids, and organic acids (Hussain et al., 2021). Fresh stone fruits are susceptible to decay, which several pathogenic fungi can cause (Lopez-Reyes et al., 2013). The main pathogens that infect peach fruits include *Botrytis cinerea* (Pers.: Fr.), *Penicillium* expansum (Link), *Rhizopus* stolonifer (Ehrenb. Fr.), and *Monilinia* spp. (Karabulut & Baykal, 2002; Zhang et al., 2007). Peach and nectarine production is stable and has been growing in recent years. The main problem is during the storage period and transportation of fruits due to their rapid decay and high transpiration rate at room temperature, which hinders their export (Lurie and Crisosto, 2005; Hussain et al., 2021). Losses caused by various fungal pathogens during fruit storage can reach up to 50% under favourable conditions; losses are significantly lower with synthetic fungicide treatments, around 10% of fruit production (Margosan et al., 1997). The global trend is to seek new alternatives to synthetic fungicides that can successfully control diseases during storage while having no side effects on human health (Johnson & Sangchote, 1994). The need to develop new methods for controlling fruit decay is driven by the excessive use of fungicides during vegetation, and the ecological plasticity of phytopathogens reduces the effectiveness of used fungicides (Lopez-Reyes et al., 2013). Improving fruit production quality by extending shelf life, reducing decay rates, and providing minimally processed fruits that are safe for human health is possible through the use of biocompatible packaging. Biological fruit packaging has been researched and developed for healthier preservation (Wu et al., 2022; Hadimani et al., 2023).

Fruit coatings are made from edible polymer materials derived from renewable sources, which are by-products of the food industry. They are used as edible packaging to extend shelf life and improve the quality of food products by adding active ingredients such as antioxidants, vitamins, and antimicrobial agents (Hosseinnejad & Jafari, 2016).

Chitosan is a natural polysaccharide biopolymer derived from chitin. Depending on the degree of deacetylation, chitosan can have several amino groups. This polycationic nature

imparts antimicrobial properties to chitosan, which facilitates interaction with negatively charged microbial cell walls and cytoplasmic membranes. These interactions lead to reduced osmotic stability, membrane disruption, and eventual leakage of intracellular components (Ma et al., 2008; Banerjee et al., 2010). Cited as the second most widespread polysaccharide in nature, chitosan possesses a range of positive qualities, including being biodegradable and non-toxic to humans and the environment, as well as biocompatible and biofunctional (Sharma et al., 2021). The biodegradation of the chitosan depends on the concentration of the nutrients and on the microorganisms in the coated product (Nakashima et al., 2005). Based on other references the chitosan is stable for the first 30 days and the process of degradation is around 100-200 days, depending on the used additives as well (Schnabl et al., 2024).

Among the polysaccharides used as edible packaging, sodium alginate (NaAlg), derived from marine brown algae, also finds application. This biopolymer is of interest as a potential coating component due to its unique colloidal properties and its ability to form strong gels alone or in combination with aqueous solutions. Alginate extends the shelf life of fruits by reducing diffusion processes and maintaining high concentrations of active molecules on the food surface. Alginate-based coatings provide additional protection to fruits by acting as a semi-permeable barrier to reduce weight loss, gas exchange, respiration, and the rate of oxidative reaction (Rojas-Grau et al., 2009). The existing literature says, the biodegradation of the alginate is much faster than the chitosan's. The weight loss of the alginate in the first 30 days is near to 50% and the fully degradation is around 45 days (Dalal et al., 2023).

This study aimed to determine the influence of the biopolymers chitosan and alginate after artificial inoculation of peach fruits with the pathogen *Monilinia* spp.

MATERIALS AND METHODS

The infected nectarine fruits were selected with visual symptoms of rot caused by fungal pathogens. A piece of infected tissue was placed on Potato Dextrose Agar (PDA) and the Petridis dish was incubated at a controlled temperature. The pathogen was isolated by standard phytopathological methods. The single colony of the pathogen *Monilinia* spp. was cultured on PDA in a controlled environment maintained at a constant temperature of 23°C, to ensure optimal fungal growth and sporulation.

Nectarine fruits of the 'Andrews' cultivar were selected as the experimental fruit. Fruits were harvested directly from the field at pre-consumer maturity, ensuring uniformity in ripeness. To eliminate surface contaminants, the nectarines were surface sterilized with 70% alcohol.

The inoculation was performed using a standardised spore culture of the *Monilinia* spp. pathogen. The spore concentration was precisely determined using a hemocytometer, adjusting the suspension to a concentration of 10^6 conidia per millilitre. A precise volume of 25µl of this spore suspension was injected into each nectarine to begin infection. The inoculation fruits were stored in a refrigerator at 4°C for 24 hours.

The biopolymers chitosan and alginate were used at a 1% concentration. The infected nectarine fruits were dipped in a solution of the biopolymers each fruit single.

The development of fruit rot was recorded dynamically every two days. This was achieved by measuring the diameter of the rot-affected area on each fruit. Measurements were conducted using a calliper to ensure the accuracy and consistency of the data. These dynamic measurements allowed tracking the progression of rot over time and evaluating the effectiveness of biopolymer coatings (chitosan and alginate) in delaying or preventing the development of the pathogen *Monilinia* spp.

For control variants were used inoculation fruits without the application of biopolymers. For data significance, 20 fruits per replicate were used for each treatment variant (control, chitosan, and alginate).

The percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Inhibition}\% = \frac{(dc - dt)}{dc} \cdot 100, \text{ where}$$

dc = the average diameter of the fungal colony in the control variant, dt = the average diameter of the fungal colony of the treatment variant. In

addition, the weight loss of uninfected fruits was measured. The values were presented as a percentage during observation.
The data obtained was analysed statistically using the SPSS 19.0 program. The data was subjected to Duncan's Multiple Range Test (MRT) at P≤0.05.

RESULTS AND DISCUSSIONS

To investigate the efficacy of biopolymer coatings on nectarines, it was necessary to isolate and cultivate the fungal pathogen responsible for the infection. Samples were isolated from infected nectarine fruits, from which the fungal pathogen *Monilinia* spp. was isolated. The isolation was performed by excising infected tissue from the fruit and placed on a potato dextrose agar (PDA) medium in a Petri dish (90 mm). The samples were incubated in a controlled environment maintained at a constant temperature of 23°C. This temperature was optimal for the growth and sporulation of *Monilinia* spp. During 10 days, colonies of *Monilinia* spp. developed on the PDA. Single colony were transferred in Petri dish to obtain a pure culture of the pathogen. This process of transferring single colonies was repeated three times that the culture was free of other contamination.
The pure culture of *Monilinia* spp. was used to inoculate nectarines. The coated biopolymers chitosan and alginate were applied 24 hours after inoculation. The control fruits, which were not coated with biopolymers, were also inoculated with the pathogen to provide a baseline for comparison. This controlled experimental assessment evaluated the inhibitory effect of chitosan and alginate on the growth of *Monilinia* spp. on nectarines.
The results (Table 1) demonstrated the growth of pathogens over ten days in nectarines application with alginate, chitosan, and an untreated control group. After two days, the infected area was relatively small in all variants, with alginate-treated fruits showing a slightly lower infection level (6.1 mm) compared to chitosan (7.8 mm) and the control (6.9 mm). By the fourth day, the infection spread further, with alginate (10.5 mm) and the control (10.2 mm) showing similar levels of fungal growth, while chitosan-treated fruits exhibited a slightly lower infection rate (8.2 mm).

Table 1. Inoculation of nectarine fruits (*Monilinia* spp.) by application with biopolymers chitosan and alginate

Variant	Infected area of the fruits (mm)				
	After two days	After four days	After six days	After eight days	After ten days
Alginate	6.1±1.68a	10.5± 5.50 a	13.2± 10.06 a	17.7 ±7.93a	21.5± 7.47 a
Chitosan	7.8 ± 2.73a	8.2 ± 4.69a	10.6± 9.00 a	13.5 ± 7.09a	12.7± 6.29 a
Control	6.9 ± 6.37a	10.2± 7.47 a	14.7± 12.51 a	21.5± 12.70 a	21.7 ± 12.20a

As the experiment progressed, differences between the variants became more evident. After six days, the control fruits showed the highest infected area (14.7 mm), while alginate (13.2 mm) and chitosan (10.6 mm) displayed lower infection levels. By the eighth day, the infection had advanced significantly in the control group (21.5 mm), whereas chitosan-treated fruits maintained a lower infection level (13.5 mm), suggesting a stronger inhibitory effect. Alginate coated fruits showed an intermediate infection level (17.7 mm).
After ten days, the control fruits had the highest infection area (21.7 mm), while alginate-treated fruits exhibited a slightly lower infection level (21.5 mm). Chitosan treated nectarines had the lowest infection area (12.7 mm), indicating its antifungal effect over time. These results confirmed that both alginate and chitosan slowed the infection, with chitosan being the more effective in limiting fungal growth on nectarines.
The statistical analysis of variance ANOVA allowed for the assessment of whether the observed differences in infection area among alginate, chitosan, and control groups were statistically significant. The result of experiment showed of the same ("a") values

that no significant differences were detected among the experimental variants. This indicated that, despite observable variations in mean infection areas, these differences were non- statistically significant.

The variability in fungal growth pathogen was calculated trought the standard deviation values.

On day 2, alginate had the lowest standard deviation (±1.68), while the control showed the highest (±6.37), indicating that infection spread more uniformly in the treated fruits. By day 4, standard deviations increased, with chitosan (±4.69) showing the least variability, suggesting that it provided a more stable antifungal effect.

The devepompent of *Monilinia* spp. in the control group showed the highest variability, reaching ±12.51on day 6 and ±12.70 on day 8, while alginate-treated fruits had slightly lower deviations (±10.06 and ±7.93, respectively). Chitosan consistently showed the lowest variation, with ±9.00 on day 6 and ±7.09 on day 8, indicating that it maintained a more uniform inhibitory effect.

By day 10, the control had the highest standard deviation (±12.20), followed by alginate (±7.47), while chitosan-treated fruits showed the most stable results (±6.29). These trends also concirmed that, although all biopolymers slowed infection, chitosan provided the most consistent protection against fungal growth.

The graph (Figure 1) displayed the dynamics of *Monilinia* spp. inhibition by alginate and chitosan across five measured in each two days of development of mycelia. The percentage of inhibition by alginate started with a negative value (-2.94%). The maximum inhibition reached 17.67% at the fourth measure but decreased to 0.92% at the last observation.

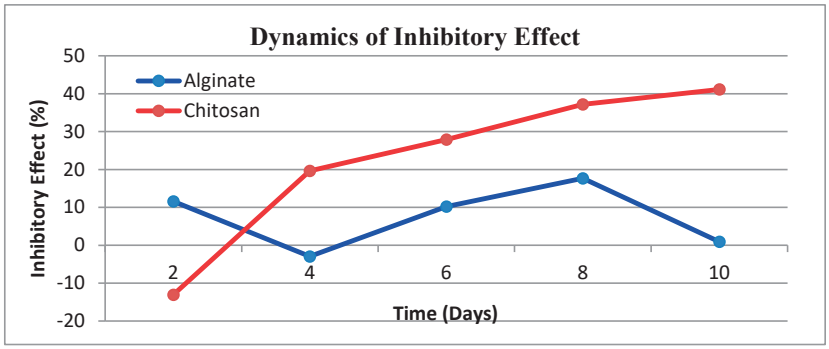


Figure 1. Inhibition of mycelia growth relative to control variant (%)

The results of edible film chitosan showed a progressive increase in inhibition, the value started with a negative percentage (-13.04%) but in the second and the next measures increased significantly (41.11%) the pressure of the pathogen. The biopolymers demonstrated more stable and effective inhibition of *Monilinia* spp. compared to alginate. Chitosan showed greater potential for controlling the brown rot caused by the fungus pathogen *Monilinia* spp.

Numerous authors have investigated the influence of biopolymers, including chitosan and alginate, on various fungal pathogens. The significant inhibition report (Romanazzi et al., 2017) of inoculation strawberries with *Botrytis cinerea* when treated with chitosan. Confirms have been reported from El Ghaouth (1992) was studied the antifungal activity of chitosan against pathogens *Botrytis cinerea* and *Rhizopus stolonifer*. The effectiveness of chitosan was reported with a coating enriched with cinnamon oil against *Monilinia fructicola* (Duan et al., 2019).

It has also been reported that alginate has a positive effect on successfully controlling pathogens during the storage period. For example, citrus fruits can be inoculated with *Penicillium digitatum* (Rojas-Graü et al., 2008), and sweet cherries can be inoculated with *Monilinia fructicola* (Gol et al., 2013).

Data obtained showed the weight loss (in percentages) of fruits treated with alginate and chitosan, as well as a control variant, the

nectarine fruits were refrigerated storage (Table 2).

The expected progressive increase in weight loss, reflecting the natural dehydration of fruits during storage (3.53%, 6.63%, 7.61%) in the control variant, was observed in a significantly lower weight loss in fruits coated with chitosan and alginate compared to the control group fruits. This indicated that the chitosan and alginate coating effectively reduced the rate of dehydration, by creating a barrier that limited moisture transfer, which was confirmed by the data in Table 2, and was consistent with the research of the author (Momchilova, M. et al., 2021).

Table 2. Relative weight loss of nectarine fruits covered with different edible coatings during storage

Variant	Weight Loss (%)				
	I	II	III	Sum	Average SD
Alginate	3.02±0.37	1.94±0.22	3.80±0.50	8.77	±0.37
Chitosan	5.73±0.79	2.76±5.03	1.36±4.35	9.85	±3.39
Control	3.53±7.43	6.63±5.43	7.61±1.55	17.77	±4.80

*SD - Standard Deviation

Edible films prevented moisture losses during postharvest storage therefore, water vapour uptake and water vapour permeability were important parameters to characterize the biopolymers used. Water vapour uptake of chitosan films decreased during storage at room temperature but increased during storage at low temperatures in a freezer and refrigerator. Thinner chitosan films exhibited lower water vapour permeability (Kerch G. & Korkhov V., 2011). Chitosan coatings, effectively delay respiration, reduce weight loss, and extend the shelf life of fruits and vegetables during postharvest storage. Chitosan-based edible coatings impact shelf life, microbiological quality, and biochemical processes, supplementing previous reviews (Dhall, 2013; Shiekh et al., 2013). Alginate treatment was an effective tool to delay the postharvest ripening process of sweet cherries. This resulted in reduced color changes, losses in acidity and firmness, and respiration rate. Furthermore, alginate treatment maintained a higher concentration of total phenolics (Díaz-Mula et al., 2012). The combination of thymol with sodium alginate films offers an alternative to traditional biocompatible wrap for packaging fresh-cut fruits. This composite film holds promise, but its practical implementation at an industrial level necessitates further advancements and optimization (Chen et al., 2021).

The fruits cover with alginate, the standard deviation across different storage periods was relatively low (±0.37) on the fourth day, the value ±0.22 on the sixth day, and 0.50 on the eighth day. The lowest value (±0.22) on the sixth day indicated minimal variation in weight loss measurements, suggesting that alginate provided a uniform and stable effect on moisture retention in the fruit. The slightly higher deviations on the fourth and eighth days still remained low, implying that alginate acted consistently across all samples, reducing water loss.

In contrast, nectarines treated with chitosan exhibited much higher standard deviations ± 0.79 on the fourth day, ± 5.03 on the sixth day, and ±4.35 on the eighth day. While the fourth day showed relatively low deviation, indicating some stability, the sixth and eighth days demonstrated significantly greater variation. The high deviations (±5.03 and ±4.35) suggested that chitosan coating wasn't uniform and infected area of fruits were measured with high variation.



Figure 2. Nectarine fruits application with alginate

The control group, which was not treated with any coating, exhibited the highest standard deviations, indicating extremely large differences in weight loss among individual fruits. On the fourth day, the standard deviation reached ±7.43 the highest recorded value in the study suggesting that while some samples retained moisture, others lost significant amounts of water. On the sixth day, the deviation decreased slightly to ±5.43 but remained high, while on the eighth day, it dropped further to ±1.55, suggesting that over time, the rate of weight loss became somewhat more uniform, although still more variable than in the treated groups.

The total weight loss of 17.77% emphasizes the need for protective coatings to extend the shelf life of nectarines.

Chitosan results showed a larger initial weight loss (5.73%) compared to alginate and the control variant. This may be due to the slower formation of a protective layer by chitosan or its greater permeability in the early stages of storage. However, weight loss decreases significantly during the second (2.76%) and third (1.36%) periods, indicating that chitosan becomes more effective over time. The total weight loss of 9.85% was higher than that of alginate but still significantly lower than the control group. The effectiveness of biopolymers chitosan and alginate was confirmed in many researches (Gol N. B., 2013; Petri S. & Sharma R. R., 2021).



Figure 3. Nectarine fruits application with chitosan

Comparing the average standard deviations across the three treatment ±0.37 for alginate, ±3.39 for chitosan and ±4.80 for the control varint it was evident that alginate provided the most stable coating, ensuring minimal variation in weight loss. Chitosan showed greater variability, potentially due to uneven distribution or interactions with the fruit surface, while the control group experienced the most unpredictable weight loss, confirming the lack of a protective barrier.

The coating fruits with alginate showed effectiveness in reducing weight loss. The low weight loss during the first two periods of measuring (3.02%, 1.94%) indicates that alginate quickly forms an effective barrier that slowed down moisture evaporation. The total weight loss of 8.77% is significantly lower than the control variant, confirming the protective properties of alginate.

These findings aligned with previous scientific studies analyzing the effects of coatings on fruit water loss. For example, research by Valero et al. (2013) on plums treated with chitosan also reported high standard deviations in later storage stages, attributed to uneven coating distribution and varying water loss rates. Olivas & Barbosa-Cánovas (2005) found that alginate coatings on apples and pears significantly reduced standard deviation in weight loss, forming a more homogeneous barrier against evaporation. Similarly, Dhall (2013) reported that control samples without coatings exhibited the highest deviations, consistent with observations in the present study.

CONCLUSIONS

The chitosan coating demonstrated the least mycelia growth of inoculated nectarines, although the differences compared to the other variants were not statistically significant.

The best variant of inhibition of mycelia growth reached 41.11% at the end of the observation period, compared to the alginate which was measured at 0.92%.

The biopolymer alginate was most effective in reducing weight loss.

Based on the experimental results and on the biodegradability of the applied biopolymers both of them were capable of short time fruit storage but with different time stability and other advantages.

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VEGETATIVE AND REPRODUCTIVE POTENTIAL OF BLACKBERRY CULTIVARS "HULL THORNLESS" GROWN IN DIFFERENT REGIONS OF BULGARIA

Stanislava ATANASOVA[1], Diyan GEORGIEV[2, 3], Maria GEORGIEVA[2, 3]

[1]Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
[2]Agricultural Academy, Sofia, Bulgaria
[3]Research Institute of Mountain Stockbreeding and Agriculture, 5600, Troyan, Bulgaria

Corresponding author email: stanislava.atanasova@trakia-uni.bg

Abstract

The vegetative and reproductive traits of the blackberry cultivar Hull Thornless, grown in different agro-ecological conditions of Bulgaria, was monitored. The study was conducted during the period 2022-2024 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan and the Experimental Field at the Faculty of Agriculture of Trakia University - Stara Zagora. The indicators of average number, average height (m) and thickness of shoots (mm) of two linear meters of the inter-row area were monitored. The results show that the studied parameters were influenced by environmental factors during the study. Regarding vegetative indicators, the number of shoots (17 ± 6.34), the thickness of the shoots (14.85 mm ± 2.36) and the height of the shoots (3.49 m ± 0.32) are greater in the blackberry grown in the conditions of Troyan. During the tested period, the highest average yield (1666.33 g/2m) and average fruit weight (5.63 g) was recorded by the plants in the plantation of Stara Zagora.

Key words*: berry fruits, fruit weight, vegetative indicators, yield.*

INTRODUCTION

The blackberry belongs to the genus *Rubus, subgenus Eubatus,* family *Rosaceae.* The genus *Rubus* includes 13 subgenera distributed worldwide (GRIN datebase, USDA, 2006), of which five are of greatest breeding importance - *Idaeobatus* - raspberry, *Eubatu*s - blackberry, *Anoplobatus* - decorative raspberry, *Cyclactis* - northern grassy raspberry and *Chamaemorus* - wild raspberry (Hollman et al, 1996; Bravo et al., 1998; Sellappan et al., 2002; Wang et al., 2000; Wada et al., 2002). The blackberry cultivars developed so far are derived from 10 American and 3 European species. Today, many high-quality and high-yielding cultivars are used in blackberry production (Lykins et al., 2021), yet the industry needs new cultivars that adapt to changing climatic conditions and provide higher yields to meet modern requirements. Blackberries (*Rubus fruticosus* L.) are widespread throughout the world, but areas with mild winters and prolonged temperate summers are more suitable for their cultivation. The main production areas are North America, South America, Central America, Europe, Asia, and Africa.

In recent years, Europe has become one of the world leaders in blackberry production.

The largest areas of this crop have been recorded in Serbia and Hungary (Cuevas-Rodríguez et al., 2010; Paczkowska-Walendowska et al., 2021; Skrovankova et al., 2015; Zhao, 2007; Zia-Ul-Haq et al., 2014). In Serbia, after raspberries, blackberries are the most widespread small-fruited species and occupy an area of 4 000 ha, with production mainly concentrated in the central, northwestern and southern regions. The dominant cultivars are Čačanska Bestrna and Thorn Free, which represent for 95% of blackberry production in the country (Nikolić & Milivojević, 2010).

Due to increasing awareness of the valuable qualities of the fruit, global consumption of berry and berry-based products has increased significantly (Nile & Park, 2014; Padmanabhan et al., 2016). Fruits of the genus *Rubus* are among the rich in bioactive compounds. Their nutritional profile indicates that they contain dietary fiber, vitamins, minerals and carbohydrates (Cuevas-Rodríguez et al., 2010; Kiple et al., 2000; Vega et al., 2021).

Blackberries are present on the market for fresh consumption, but are more often processed into juices, jams, purees, concentrates and sweets. They are notable for their health benefits due to their high dietary content of fiber, vitamin C, vitamin K, folic acid and the essential mineral manganese (Sariburun et al., 2010). The productivity of small fruit crops (raspberry, blackberry) is determined by the number of shoots per meter, their development (height and thickness) and the number and weight of fruits per bush.

Fruit weight is an important qualitative reproductive trait influenced by genetic background (Kaldmäe et al.,2013; Kikas et al., 2017) and yield. Yield is one of the main indicators in fruit crops, determining to a significant extent the application of the cultivar in practice (Georgiev, 2009). In their studies, a number of authors found that environmental and cultivar factors have variable effects on vegetative and reproductive characteristics of small-fruited plants.

Strik (2012) indicated that fruit bud differentiation in raspberries and blackberries depends exclusively on the light regime, temperature conditions and the cultivation system applied.

There are relatively few blackberry plants in our country. The main reasons for the poor cultivation of this fruit species are its insufficient knowledge and the lack of cold-resistant cultivars with stalk upright and without thorns.

According to world and national literature, suitable growing areas for blackberry are high mountain areas with the necessary air and soil humidity. For locations where there is insufficient rainfall, an irrigation system is necessary.

The aim of the present study is to follow the behaviour of the introduced blackberry cultivar “Hull Thornless” grown under the mountain conditions of the Troyan region and the plain conditions of the town of Stara Zagora.

MATERIALS AND METHODS

The research work was carried out in the period 2022-2024 in the experimental field of Trakia University - Stara Zagora and the collection plantation of RIMSA - Troyan. The object of the research is the blackberry cultivar “Hull Thornless”, introduced in the USA, which has not yet been sufficiently tested for our climatic conditions.

The blackberry plants were planted at 3.00 m row spacing and 1.50 m row spacing in two variants:

I var. - Plants planted in the collection plantation of RIMSA - Troyan;

II var. - Plants planted in the experimental field of Trakia University- Stara Zagora.

The trial was set in three replicates, each two linear metres of in-line area. A support structure was constructed. The soil in the area of Troyan is light grey forest, gleyey, medium to heavy sandy loam, moderately eroded, with low humus content (Mihailova at al., 2008), and in Stara Zagora the soil is meadow-clay, medium sandy loam, with a slightly alkaline reaction.

The following indicators are monitored:

- Number of shoots per two linear meters - the number of shoots in each replicate was listed;
- Shoot height measured in (cm) - all shoots from each replicate were measured;
- Shoot thickness (mm) - measured at 10 cm from the soil surface;
- Average fruit weight (g) - samples of 30 fruits were taken at one of the first and two of the mass harvests;
- Average yield (g/2m).

The methodology for studying plant resources in fruit plants (Nedev et al., 1979) was used to report the indicators. The data were processed by variance-statistical univariate, bivariate analysis of variance and correlation analysis (Lidanski, 1988), MS Excel - 2010 software was used.

RESULTS AND DISCUSSIONS

The shoot-forming ability is an important factor characterizing the potential of the cultivar as well as determining to a large extent the cultivation technology. The results of the first trial showed a large difference between the cultivar variants in the index, which ranged from 4.33 pcs (variant II) to 17.00 pcs (variant I) (Table 1). The average shoot height of the blackberry plants of variant I (275.92 cm) was found to be higher. For the average shoot thickness parameter, the differences in values

for both cultivation treatments were less and ranged from 8.21 mm (treatment II) to 12.92 mm (treatment I). In 2016, Karaklajić-Stajić, Ž et al. reported an average number of shoots ranging from 3.41 to 5.19 pcs, results lower than our study. The same authors reported twice as high a value for the average shoot length (353.60 and 530.22 cm) and diameter between 13.89 and 18.09 mm. Regarding the reproductive index, the average fruit weight in both locations did not show significant differences, 4.54 g (variant I) and 5.08 g (variant II). In the first experimental year, the yield obtained in the second variant of the tested cultivar was higher and was 2018.40 g /2m.

Table 1. Vegetative and reproductive indicators in "Hull Thornless" cultivar for the period of 2022-2024

Cultivar / Indicators	I variant	II variant
2022		
Average number of shoots/m	17.00	4.33
Average height of shoots (cm)	275.92	180.36
Average shoots thickness (mm)	12.92	8.21
Average fruit weight (g)	4.54	5.08
Average yield (g/2m)	918.08	2018.40
2023		
Average number of shoots/m	17.00	5.33
Average height of shoots (cm)	266.19	331.04
Average shoots thickness (mm)	12.73	8.29
Average fruit weight (g)	4.59	6.84
Average yield (g/2m)	781.88	1469
2024		
Average number of shoots/m	12.33	7.33
Average height of shoots (cm)	348.92	284.33
Average shoots thickness (mm)	14.85	6.61
Average fruit weight (g)	4.16	4.97
Average yield (g/2m)	2298.65	1511.62

In the second year of the trial, the average number of shoots in the first variant of the cultivar was the same compared to the previous year (17 pcs) (Table 1). In the second variant a higher number of shoots was recorded compared to the beginning of the trial (5.33 pcs). The average height of shoots ranged from 266.19 cm (variant I) to 331.04 cm (variant II). The average shoot thickness of the blackberry cultivar grown in Troyan was greater at 12.73 mm. The average fruit weight in the second year in variant I was very similar to the previous year (4.59 g), while in variant II it was higher at 6.84 g. In 2023, a lower average yield was recorded for both variants compared to the previous year - 781.88 g/2m (variant I), 1469 g/2m (variant II). In the third year, the number of shoots of the first variant of the tested cultivar decreased significantly to 12.33, but an opposite trend was observed in terms of average height and thickness of shoots. In the second variant, the number of shoots increased and reached 7.33 counts (Table 1). but a significant decrease in shoot height and thickness was found. In 2024 trial year, significantly higher average yield was recorded in first variant of the tested cultivar 2298.65 g/2m which was also found to be the highest during the trials period. A yield of 1511.62 g/2m was recorded for the second variant. There were no significant differences in the average fruit weight in both locations of cultivation, but the advantage, although minimal, was in the second variant. In their study on the vegetative traits of the blackberry cultivar "Čačanska Bestrna" (Karaklajić-Stajić et al., 2016), found that the indicators, number of stems (4.48 ± 0.21) and stem height (467.91 ± 16.26 cm), were greater in blackberry grown with a rain protection system, while stem diameter (15.68 ± 0.45 mm) was greater when grown outdoors.

In the first year of the trial, a very strong positive correlation between shoot number and shoot height and a strong correlation between yield, shoot number and average shoot height was observed in the cultivar in the first variant (r=0.99, r=0.77 and r=0.81, respectively)

(Table 2). Significant negative correlation was recorded between yield and shoot thickness (r= -0.70). Other correlations for the remaining parameters were observed in the second variant, with a strong negative correlation reported between number, shoot thickness and yield (r= -0.73, r= -0.84), a strong positive correlation between yield, average shoot height (r=0.82) and a very strong correlation between average shoot thickness and height, and between yield and shoot thickness (r=0.91 and r=0.98).

Table 2. Correlation dependences between vegetative and reproductive indicators of "Hull Thornless" in 2022

Indicators	Average number of shoots /m	Average height of shoots (cm)	Average shoots thickness (mm)	Average yield (g/2m)
I variant				
Average number of shoots/m	1			
Average height of shoots (cm)	0.99	1		
Average shoots thickness (mm)	-0.08	-0.14	1	
Average yield (g/2m)	0.77	0.81	-0.70	1
II variant				
Average number of shoots/m	1			
Average height of shoots (cm)	-0.38	1		
Average shoots thickness (mm)	-0.73	0.91	1	
Average yield (g/2m)	-0.84	0.82	0.98	1

The statistical correlation between the indicators of the cultivar "Hull Thornless" in 2023 from the trial at planting variant I showed a very strong positive correlation between the number of height and shoot thickness and between the average height and shoot thickness, with a correlation coefficient of (r=0.99), respectively (Table 3). No correlation was observed among the other parameters. In blackberry cultivation variant II, a very strong negative correlation was observed only between the indicators height and shoot thickness (r=0.99) and a high positive correlation between shoot thickness and yield (r=0.77). For all other parameters, a weak to significant correlation was observed (r<0.7).

In the third trial year, the first cultivar variant showed a very strong correlation between shoot thickness and height with a correlation coefficient of (r=0.99) and a high but negative correlation between average height, average thickness and yield with a correlation coefficient of (r=-0.92, r=-0.91) (Table 4).

Table 3. Correlation dependences between vegetative and reproductive indicators of "Hull Thornless" in 2023

Indicators	Average number of shoots /m	Average height of shoots (cm)	Average shoots thickness (mm)	Average yield (g/2m)
I variant				
Average number of shoots/m	1			
Average height of shoots (cm)	0.99	1		
Average shoots thickness (mm)	0.99	0.99	1	
Average yield (g/2m)	0.09	-0.02	0.02	1
II variant				
Average number of shoots /m	1			
Average height of shoots (cm)	0.57	1		
Average shoots thickness (mm)	-0.52	-0.99	1	
Average yield (g/2m)	0.22	-0.67	0.71	1

For all other parameters, weak to moderate correlation was observed (r<0.5). In variant II of plant growth, a very high correlation was found only between the number of shoots and their height (r=0.99). A high correlation was observed between the parameters number, height and thickness of shoots, respectively (r=0.89, r=0.85). A weak to moderate correlation was observed for all other indicators (r<0.5).

Table 4. Correlation dependences between vegetative and reproductive indicators of "Hull Thornless" in 2024

Indicators	Average number of shoots /m	Average height of shoots (cm)	Average shoots thickness (mm)	Average yield (g/2m)
I variant				
Average number of shoots/m	1			
Average height of shoots (cm)	0.43	1		
Average shoots thickness (mm)	0.46	0.99	1	
Average yield (g/2m)	-0.05	-0.92	-0.91	1
II variant				
Average number of shoots /m	1			
Average height of shoots (cm)	0.99	1		
Average shoots thickness (mm)	0.89	0.85	1	
Average yield (g/2m)	-0.03	0.07	-0.47	1

The averaged values of vegetative and reproductive indicators of the studied blackberry cultivar over the three-year period reported higher shoot number (15.44), and shoot thickness (13.65 mm) scores of I variant and shoot height (414.91 cm), average yield (1666.33 g) and average fruit weight (5.63 g) of II variant (Table 5). In the analysis of variance, it was observed that on the parameter of average number of shoots, the factors of variance (67.67%) and random factors (20.53%) were the most influential and the interaction between years (0.16%) was the least. In terms of average height of shoots, the years during the test period (22.44%) and random factors (73.17%) were the most influential.

Table 5. Average values of vegetative and reproductive indicators by variants in "Hull Thornless" for the period (2022-2024)

Cultivar / Indicators	I variant Troyan	II variant Stara Zagora	Level of significance (%)/ Degree of influence of factors (%)
Average number of shoots/m	15.44	5.42	(P<0.05)
			A=0.16
			B=67.67
			C=11.63
			D=20.53
Average height (cm)	296.61	414.91	(P<0.05)
			A=22.44
			B=4.14
			C=0.25
			D=73.17
Average shoots thickness (mm)	13.50	6.45	(P<.05)
			A=0.02
			B=85.04
			C=7.01
			D=7.93
Average yield (g/2m)	1332.87	1666.33	(P<0.05)
			A=10.67
			B=0.75
			C=8.39
			D=80.18
Average fruit weight (g)	4.43	5.63	(P<0.05)
			A=3.30
			B=34.96
			C=0.12
			D=61.62

Legend: A - degree of impact of years (%); B - degree of impact of the variants (%); C - degree of interaction impact of the variants and years (%); D - Degree of impact of random factors (%)

The influence of location was low (4.14%). Average shoot thickness was most strongly influenced by variants (85.04%) and significantly lower by variants and fits (7.01%), random factors (7.93%), and years (0.02%). Among the reproductive indicators, average yield was significantly influenced by years (10.67%) and random factors (80.18%). Mathematically, the differences were not proved at significance level ($P<0.05$). In the analysis of variance, it was reported that the parameter average fruit weight with the greatest influence were the variants (34.96%) and random factors (61.62%).

Linear regression was applied to quantify the relationships between vegetative indicators (number of shoots, shoot height and shoot thickness) and the reproductive indicator (yield). In 2022, the linear regression analysis performed on the test cultivar showed high correlation between vegetative traits shoot number and shoot height in variant I (R2=0. 99) and between shoot height, shoot thickness and shoot yield and shoot thickness in variant II (R2=0.82; (R2=0. 99) (Figures 1, 2, 3).

The regression analysis for the cultivar Hull Thornless in 2023 showed a high positive correlation between the number, height and thickness of shoots and between the height and thickness of shoots in the first cultivation variant. and between the height and thickness of shoots in the second cultivation variant.

The coefficient of determination R2 was high with values ranging from 0.98 to 0.99 (Figures 4, 5, 6, 7). The linear regression analysis done in 2024 between vegetative and reproductive indicators of blackberry cultivar showed high correlation between shoot height and shoot thickness in variant I (R2=0.99), and between shoot number and height and shoot number and thickness in variant II (R2=0.99; R2=0.80) (Figures 8, 9, 10).

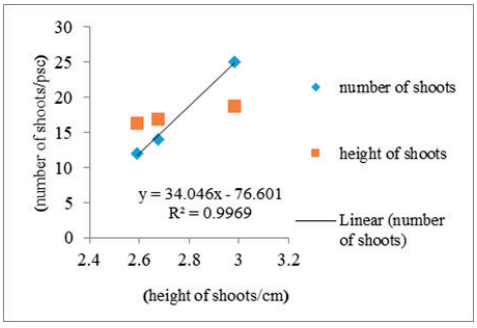


Figure 1. Regression analysis between vegetative indicators of "Hull Thornless", I variant in 2022

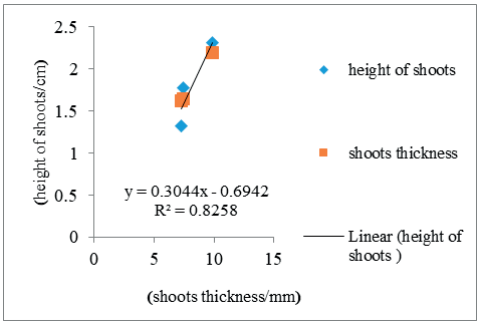


Figure 2. Regression analysis between vegetative indicators of "Hull Thornless", II variant in 2022

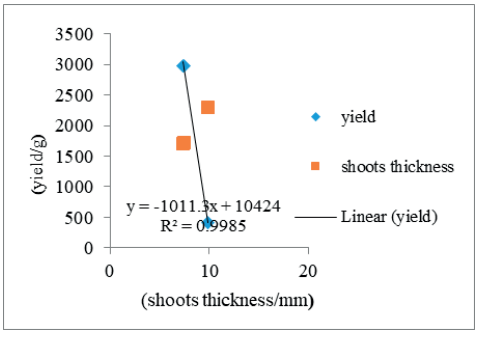


Figure 3. Regression analysis between vegetative and reproductive indicators of "Hull Thornless", II variant in 2022

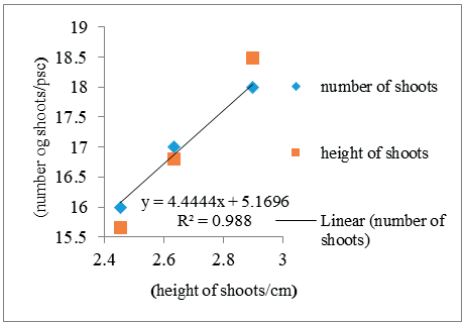


Figure 4. Regression analysis between vegetative of "Hull Thornless", I variant in 2023

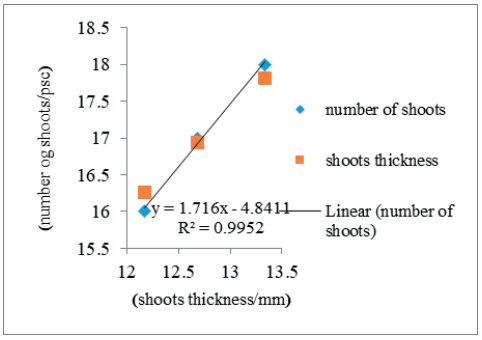


Figure 5. Regression analysis between vegetative indicators of "Hull Thornless", I variant in 2023

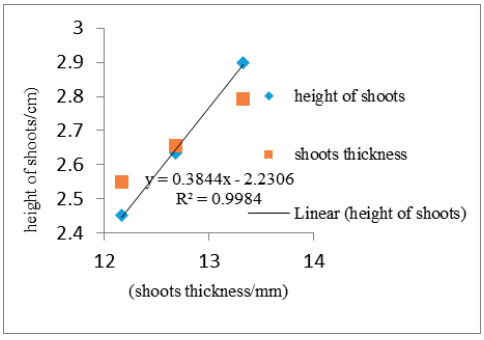


Figure 6. Regression analysis between vegetative indicators of "Hull Thornless", I variant in 2023

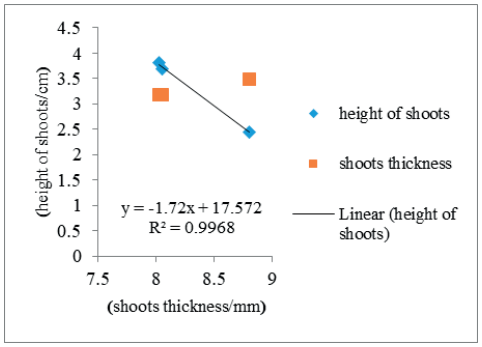


Figure 7. Regression analysis between vegetative indicators of "Hull Thornless", II variant in 2023

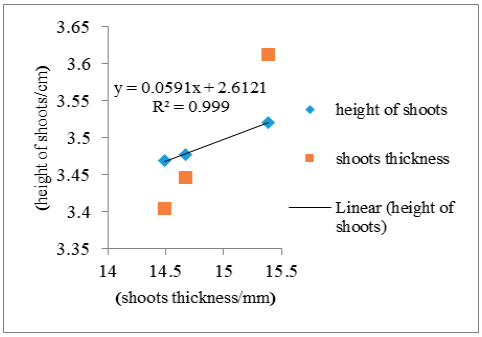


Figure 8. Regression analysis between vegetative indicators of "Hull Thornless", I variant in 2024

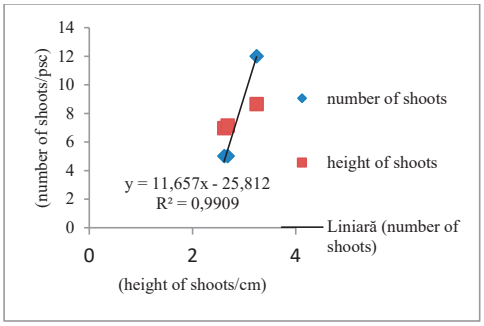


Figure 9. Regression analysis between vegetative indicators of "Hull Thornless", II variant in 2024

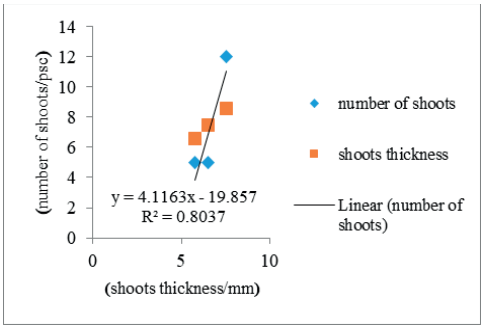


Figure 10. Regression analysis between vegetative "Hull Thornless", II variant in 2024

CONCLUSIONS

The averaged values of vegetative and reproductive indicators of the studied blackberry cultivar for the three-year period reported the higher shoot number (15.44) and shoot thickness (13.650 mm) of I variant and shoot height (414.91 cm), average yield (1666.33 g/2m) and average fruit weight (5.63 g) of II variant.

From the results of correlation relations of vegetative and reproductive traits in variant I of cv. "Hull Thornless", a very strong positive correlation was observed between average number and height of shoots in 2022 and 2023, a very strong correlation relation between number, height and thickness of shoots in 2024. In the II cultivation variant, a strong to very strong correlation was found between the studied parameters (2022), a very strong

correlation between shoot height and shoot thickness (2023) and a strong correlation between shoot number and shoot thickness to a very strong correlation between shoot number and shoot height (2024). From the results on the regression dependences of vegetative and reproductive plant traits we can summarise:
- in variant I, a very strong correlation was reported between number and height; height and shoot thickness for 2022; number and height and shoot thickness for 2023 and between height and shoot thickness for 2024.
- option II shows a strong correlation between shoot height and thickness and yield for 2022, between height and thickness for 2023 and 2024 and between number, height and thickness of shoots for 2024.

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DETECTION OF PHYTOPLASMA INFECTIONS IN VARIOUS FRUIT TREES IN BULGARIA, 2018-2024

Zhelyu AVRAMOV[1], Iliyana ATANASOVA[1], Dora PANAYOTOVA[2]

[1]Univesity of Forestry, 10, Kliment Ohridski Blvd, Sofia, Bulgaria
[2]Central Laboratory for Plant Quarantine, 120 Nikola Moushanov Blvd., Sofia, Bulgaria

Corresponding author email: iatanasova@ltu.bg

Abstract

This article covers research conducted between 2018 and 2024 aimed at detecting phytoplasma infections in various fruit crops in Bulgaria. Phytoplasma diseases, such as 'Candidatus Phytoplasma mali', 'Candidatus Phytoplasma pyri', and 'Candidatus Phytoplasma prunorum', represent a serious threat to agriculture, leading to significant losses in yields and fruit quality. The studies described in the article include the diagnosis of phytoplasma infections in different fruit crops. Molecular methods, including PCR (Polymerase Chain Reaction) and RT-RFLP analysis, were performed to identify pathogens, enabling precise and rapid detection of phytoplasmas in affected plants. More than 3,294 samples were analyzed, of which 4 tested positive for 'Candidatus Phytoplasma prunorum' and 6 for 'Candidatus Phytoplasma pyri', while 'Candidatus Phytoplasma mali' was not detected. The article emphasizes the importance of early detection and management of phytoplasma infections in fruit growing to minimize economic losses and ensure sustainable fruit production in the future.

Key words*: Phytoplasma infections, Fruit trees, Bulgaria*

INTRODUCTION

Pomiculture is one of the main sectors in agriculture, playing a key role in the Bulgaria's economy, ensuring food security, and improving the quality of life.

Among the most widely grown fruit plants in the country are apples, plums, cherries, apricots, peaches, and strawberries. These crops not only satisfy the domestic market but are also exported abroad, contributing to the increase in agricultural product exports.

The yields in horticulture depend on numerous factors, including climatic conditions, the technologies used, soil quality, and the application of modern agronomic practices.

In recent years, the areas occupied by apple orchards have remained relatively stable, ranging between 12 and 15ha. Apple yields vary significantly depending on climatic conditions. In years with favorable conditions (such as 2019 and 2022), yields reached 150,000–160,000 tons. In 2021, yields were lower (around 120,000 tons) due to spring frosts and drought.

The areas occupied by pears are relatively small in Bulgaria, ranging between 2 and 3ha. There is a slight downward trend in these areas, as pears are a less popular crop compared to apples and plums. Pear yields range between 10,000 and 15,000 tons annually.

Plums are one of the most widely grown fruit crops in Bulgaria. The areas occupied by plum orchards are around 20–25ha. There has been a slight decrease in these areas in recent years, as some old orchards have been abandoned. Plum yields vary significantly, ranging from 50,000 to 90,000 tons annually. In 2020, yields were lower (around 50,000 tons).

The areas occupied by apricots are around 5-7 ha. There has been a slight decrease in these areas, as apricots are more sensitive to climate changes and diseases. Apricot yields range between 15,000 and 25,000 tons annually.

The areas occupied by peaches range between 5 and 7ha during the period 2020–2023. A slight decrease in these areas has been observed due to their sensitivity to climate changes and diseases, making them less attractive to producers. In years with favorable conditions (such as 2022), yields reached 25,000–30,000 tons (Agrostat, 2021).

Fruit trees from the Rosaceae family can be seriously affected by phytoplasmas from the Apple Proliferation (AP 16SrX group). The AP group includes 'Candidatus Phytoplasma mali', which causes apple proliferation (AP),

'Candidatus Phytoplasma prunorum', associated with European stone fruit yellows (ESFY), and '*Candidatus* Phytoplasma pyri', associated with pear decline (PD) (Seemüller & Schneider, 2004; Marcone et al., 2010). These are among the most economically important diseases in many fruit-growing regions of Europe, as well as in Bulgaria.

Phytoplasmas belong to the class Mollicutes and are considered putative pathogens of the so-called "yellows diseases," which affect at least 1,000 plant species worldwide (McCoy et al., 1989; Seemuller et al., 2002; Trivellone and Dietrich, 2020). These microscopic, intracellular prokaryotes colonize the phloem of infected host plants, as well as various tissues and organs of their insect vectors.

Phytoplasmas are transmitted after a latent period of several days to months to plants during the feeding of their vectors, primarily cicadas and leafhoppers from the order *Hemiptera* (IRPCM, 2004; Thébaud et al., 2009).

Infection by '*Ca.* P. mali' occurs primarily in plants of the genus *Malus*, but it has also been sporadically detected in other non-typical host plants, such as stone fruits, European pear (*Pyrus communis*), and Asian pear (*Pyrus pyrifolia*) (Lee et al., 1995; Del Serrone et al., 1998; Seemüller & Schneider, 2004; Mehle et al., 2007). '*Ca.* P. pyri' is mainly associated with the genus *Pyrus* (Seemüller & Schneider, 2004). On the other hand, '*Ca.* P. prunorum' causes economically significant diseases in apricot (*Prunus armeniaca*), Japanese plum (*Prunus salicina*), and peach (*Prunus persica*) (Carraro & Osler, 2003). European plum (*Prunus domestica*), as well as some wild species of the genus *Prunus* (*Prunus spinosa, Prunus cerasifera, Prunus insititia*), are also susceptible to infection but typically do not show symptoms, making them hidden sources of infection (Carraro et al., 1998a, 2004; Carraro & Osler, 2003).

Leafhoppers play a significant role in the spread of phytoplasmas from the AP group (Tedeschi & Alma, 2004).

'*Ca.* P. prunorum' is transmitted to plants of the genus *Prunus* by the insect vector *Cacopsylla pruni* (Carraro et al., 1998b). Additionally, the cicada *Asymmetrasca decedens* (also known as *Empoasca decedens*) is considered a potential carrier of this phytoplasma (Pastore et al., 2004).

'*Ca.* P. pyri' is spread by two main vectors, *Cacopsylla pyricola* (Davies et al., 1992) and *Cacopsylla pyri* (Carraro et al., 1998c).

Among the known leafhopper vectors of *'Ca.* P. mali' are *Cacopsylla picta* (also called *Cacopsylla costalis)* (Frisinghelli et al., 2000; Jarausch et al., 2003) and *Cacopsylla melanoneura* (Tedeschi & Alma, 2004).

In addition to leafhoppers, other insects are also associated with the transmission of '*Ca.* P. mali', such as *Philaenus spumarius*, *Artianus interstitialis* (Hegab & El-Zohairy, 1986), and *Fieberiella florii* (Krczal et al., 1988).

Phytoplasmas, which cause significant economic damage to fruit trees, are widely distributed.

Pear decline was first reported in North America in the late 1940s but is believed to have European origins (Seemüller & Schneider, 2004). The infection is widespread in almost all EU countries.

'*Ca.* P. mali' has been reported in the EPPO region, (Tunisia, Syria), and North America (USA and Canada). ESFY (European stone fruit yellows) is also a widely spread infection in the EPPO region. The disease occurs with varying frequency in each country. ESFY is a serious problem in countries bordering the Mediterranean Sea (Spain, France, Italy, the Balkans), where the cultivation of susceptible and sensitive species of the genus Prunus is widespread (Steffek et al., 2012).

In Bulgaria, '*Ca.* P. prunorum' and '*Ca.* P. pyri' were first detected near the city of Plovdiv (Topchiiska et al., 2000; Topchiiska & Sakalieva, 2001, 2002).

In 1960, Apple proliferation was reported in Bulgaria by Sen and Jun Blattny, as cited by Trifonov (1972).

In 2012, the first summarized results from the official phytosanitary monitoring program conducted between 2007 and 2011 by the Bulgarian Food Safety Agency were published (Etropolska & Laginova, 2012).

The summarized results show that AP was detected in the regions of Blagoevgrad, Kyustendil, Pazardzhik, Plovdiv, and Veliko Tarnovo. PD was identified in 10 regional directorates of BFSA, including Blagoevgrad, Kyustendil, Pazardzhik, Plovdiv, Veliko Tarnovo, Sliven, Silistra, Burgas, Haskovo, and Yambol. ESFY was detected in 6 regions –

Pazardzhik, Lovech, Sliven, Dobrich, Burgas, and Yambol.

At that time, to control the spread of phytoplasmas, over 200 infected mother trees, more than 5,000 first-year nursery trees, and over 22,000 second-year nursery trees were uprooted and destroyed under the supervision of phytosanitary inspectors. In infected orchards, more than 0,2ha were destroyed (Avramov et al, 2013).

In 2016, an analysis for the presence of phytoplasmas was conducted in the Kyustendil region of Bulgaria. The results showed that diseases from the AP group were present in 85.0% of the tested apple trees, 62.0% of the tested pear trees, and all tested plum trees, along with the observation of many symptomatic trees. This indicates a high prevalence of phytoplasmas in and around the studied orchards in the Kyustendil region (Borisova & Kamenova, 2016).

At present, these phytoplasma diseases have had their status changed in Bulgaria from quarantine to non-quarantine regulated pests.

Additionally, all species of the genus *Cacopsylla* were described in Bulgaria by entomologists as early as the 1960s (Harizanov, 1966a, 1966b, 1982), long before they were recognized as vectors of phytoplasmas.

In 2011, the first report of the detection of '*Ca.* P. prunorum' through PCR analysis in individuals of the vector *C. pruni,* collected from Sofia and Kyustendil, was published (Etropolska et al., 2011a).

During a study conducted between 2011 and 2013, AP, ESFY, and PD were detected in commercial or non-cultivated orchards in five of the most important fruit-growing regions of Bulgaria: Sofia, Blagoevgrad, Plovdiv, Kyustendil, and Sliven regions (Etropolska et al., 2015).

The main symptoms of AP disease in apples, as described by Nemeth (1986), include "witches' brooms," enlarged stipules, small and poor-tasting fruits, and reddish discoloration of the leaves. The fruits of infected trees are deformed and smaller in size compared to those of healthy trees.

In pear trees, slow growth is observed. The leaves are fewer in number, smaller, light green, and have curled edges. The growth of shoots is very weak. The most characteristic symptom of PD infection is the reddish discoloration of the leaves and their premature fall in early autumn.

The symptoms described for ESFY include early bud development, followed by reddish discoloration and curling of the leaves, which are smaller in size (Carraro et al., 1992; Jarausch et al., 2000a; 2000b; Carraro and Osler, 2003).

There is no evidence of transmission through seeds or pollen (Seidl & Komarkova, 1974). *Cuscuta sp*. can transmit '*Ca*. P. mali' between apple, *Catharanthus roseus*, and other hosts.

To date, only hawthorn (*Crataegus sp*.) has been identified as a wild plant reservoir in the pathogen's epidemic cycle (Janik et al., 2020).

Over longer distances, *'Ca.* P. mali' is mainly spread through the movement of infected trees, cuttings, and rootstock material of apples.

'Ca. P. prunorum' is easily transmitted both in summer and winter (Riedle-Bauer et al., 2012).

Imported plant material is considered the primary way of introducing '*Ca*. P. prunorum' into Turkiye (Ulubaş Serçe et al., 2006). The local spread of '*Ca*. P. prunorum' is primarily driven by insect vectors and infected *C. pruni* can transmit phytoplasmas to Prunus species over distances of 27–50 km (Marie-Jeanne et al., 2020).

Due to dynamic ecological, climatic and anthropogenic factors, phytoplasma infections in fruit orchards are inconsistent.

Given the scarcity of data, this study aims to identify spreading of these infections and support future preventive measures to limit their spread in Bulgaria.

MATERIALS AND METHODS

Annually, phytosanitary inspectors from the Regional Food Safety Directorates conduct surveys across the entire country.

The focus of the research is on nurseries producing fruit planting material and high-risk fruit plantations. The species examined during this period were *Malus L., Prunus domestica, Prunus avium, Prunus cerasus, Pyrus L., Prunus armeniaca, Prunus persica* and *Cydonia* L. Samples were collected from 21 regions cover all over Bulgaria and sent to the laboratory of the University of Forestry.

In 2018, a total of 718 samples, primarily from fruit planting material, were processed and analyzed; in 2019, the number of samples was

637; in 2020 – 532 samples; in 2021 – 565 samples; in 2022 – 485 samples; in 2023 – 354 samples; and in 2024 – 116 samples, the total number of samples was 3,407 samples.

Malus L. and *Prunus domestica* were the two species with the highest number of samples in almost all years.

Prunus avium was also a frequently encountered species, particularly in regions such as Kyustendil and Plovdiv. In the past, before molecular techniques were developed, diagnosing phytoplasma diseases was challenging and primarily relied on symptom observation.

The method used to detect phytoplasmas was PCR. Total DNA was extracted from plant tissue (Doyle and Doyle, 1990) or a predetermined individual in CTAB buffer following the protocol by Marzachi et al. (1998).

Laboratory analysis was performed using Nested PCR with two pairs of universal primers, P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1996) and U3/U5 (Lorenz et al., 1995), as well as specific primers f01/r01 (Lorenz et al., 1995), followed by RT-RFLP analysis using *Rsa*I/*Alu*I restriction enzymes for final identification and species classification (Lee et al., 1998).

RESULTS AND DISCUSSIONS

Between 2018 and 2024, 3,431 laboratory analyses were conducted on 3,407 plant samples, was performed us following: for PD 541 analysis, for ESFY 2,157 analysis and for AP 733 analysis.

Phytoplasma infections were detected and confirmed in 12 number of samples. Out of all the samples examined during the period from 2018 to 2024, Apple proliferation phytoplasma was not detected in Bulgaria.

In 2018, none of the laboratory analysis showed positive reaction for the presence of phytplasmas. In 2019, positive samples for ESFY and PD were identified.

ESFY infection was detected in the Blagoevgrad region in mother trees of *Prunus domestica* (Table 2), while PD was found in the Kyustendil region in mother trees of *Pyrus communis* (Table 1).

Table 1. Confirmed PD phytoplasma infection, 2018-2024

Regions	Confirmed phytoplasma infection						
	2018	2019	2020	2021	2022	2023	2024
Total № analysis PD	**94**	**106**	**105**	**99**	**63**	**58**	**16**
Vidin	-	-	-	**PD**	-	-	**PD**
Vratsa	-	-	**PD**	-	-	-	-
Kyustendil	-	**PD**	-	-	-	-	-
Montana	-	-	-	**PD**	-	-	-
Razgrad	-	-	-	-	-	-	**PD**
Ruse	-	-	-	-	-	**PD**	-
Stara Zagora	-	-	**PD**	-	-	-	-

PD = positive result; "-"= negative result

Table 2. Confirmed ESFY phytoplasma infection, 2018-2024

Regions	Confirmed phytoplasma infection						
	2018	2019	2020	2021	2022	2023	2024
Total № analysis ESFY	**489**	**463**	**316**	**309**	**301**	**214**	**65**
Blagoevgrad	-	**ESFY**	-	-	-	-	-
Vidin	-	-	-	**ESFY**	-	-	-
Pazardjik	-	-	-	-	**ESFY**	-	-
Plovdiv	-	-	-	-	**ESFY**	-	-

ESFY = positive result; "-" = negative result

In 2020, we identified one positive sample for PD in the Vratsa region and another positive sample for PD in the Stara Zagora region (Table 1).

In 2021, the highest number of positive samples for PD were recorded - 2 positive samples in the Vidin region from fruit planting material of

Pyrus L. (Table 1), and 6 positive samples in the Montana region from *Pyrus domestica* (Table 1). We also detected one positive sample for ESFY infection in *Prunus domestica*, again in the Vidin region (Table 2).
In 2022, we identified 2 cases of ESFY in the Pazardzhik region on *Prunus persica* and in the Plovdiv region on *Prunus domestica* (Table 2).
For 2023, there was only one positive sample for PD in the Ruse region (Table 1).
During the period 2018–2024, a gradual spread of PD and ESFY phytoplasmas was observed in various regions of Bulgaria.
The highest activity of PD was in 2021, while ESFY was most active in 2022. In 2023, a significant decrease in positive samples was observed, which may be attributed to implemented control measures.
Further research and measures are necessary to limit the spread of these diseases, particularly in the Vidin, Montana, Pazardzhik, and Plovdiv regions.
After conducting laboratory analyses of all samples, according to the nature of the material, the infection found in mother trees was 1.8% of 450 samples, in fruit nurseries, the infection rate was 1.4% of 250 samples, and in functioning orchards throughout the country it was about 1% of 300 samples (Figure 1).

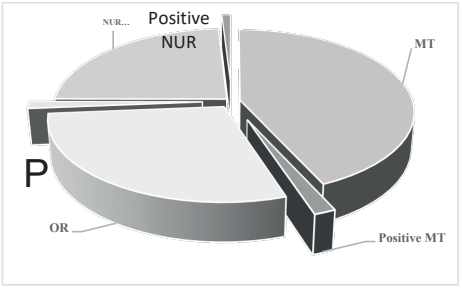


Figure 1. Spreading of infections according to testing material: MT – mothers trees, NUR – nursery, OR – orchards.

CONCLUSIONS

Fruit growing is a key sector in Bulgarian agriculture, contributing to the economy, food security, and quality of life. The most widely cultivated crops include apples, plums, cherries, apricots, peaches, and strawberries, which are also exported to international markets. Yields in fruit growing depend on climatic conditions, technologies, soil quality, and the application of modern agronomic practices.
In recent years, the areas dedicated to apple cultivation have remained stable, but yields have varied significantly depending on the climate. Phytoplasmas, which cause diseases such as Apple Proliferation (AP), Pear Decline (PD), and European Stone Fruit Yellows (ESFY), are a serious problem for fruit growing. They are primarily spread through insect vectors such as leafhoppers and psyllids.
In recent years, a gradual spread of PD and ESFY has been observed in various regions of Bulgaria, with the highest activity of PD recorded in 2021 and ESFY in 2022. In 2023, a decrease in positive samples was observed, which may be attributed to the control measures implemented.
The results were provided to the Bulgarian Food Safety Agency (BFSA). Based on these results, directives and various measures for the eradication of infections were prepared. The measures taken upon the detection of PD infection in the Kyustendil region were as follows: an additional sample was taken from the second mother tree of the same variety. Two mother trees of the "Paskresan" variety were uprooted and destroyed by burning before the results from the second tree were obtained, and a protocol was drawn up.
The additional sample from the second mother tree, which had already been destroyed, tested negative. In 2020, measures were taken in the regions of Vratsa and Stara Zagora, where infections were detected, including the destruction of fruit mother trees by the end of 2020. For 2021, the following measures were taken for the detected infections in the Vidin region for PD and ESFY: issuance of directives for the implementation of phytosanitary measures, including the purchase of healthy fruit planting material, regular treatment with approved plant protection products against vectors, strict hygiene practices such as washing and disinfecting used tools and machinery, and disinfection of pruning equipment with suitable agents (soaking in bleach or a 5-10% formalin solution or washing with a 70% alcohol solution).
In the Montana region, three "Abate Fetel" pear trees and one "Santa Maria" pear tree were

removed and burned from the mother orchard, and in the second-year nursery, 49 "Abate Fetel" pear trees and 53 potentially infected "Santa Maria" pear trees were destroyed.

Timely measures were prescribed to control the pear psyllid, and planting in the area of the destroyed trees was prohibited until the next growing season.

For 2022, upon detection of infections, the same measures as the previous year were taken: uprooting and destruction by burning, treatments to control the vector, and the use of grafts from healthy mother trees during grafting. In 2023, in the Ruse region, where a PD infection was detected, a ban was imposed on the collection of grafts, and the infected trees were removed from the nursery's varietal composition.

In 2024, we identified 2 positive samples for PD - one in Vidin district and one in Razgrad district. Despite the decrease in positive samples in 2023-2024, continued monitoring and research are necessary, particularly in regions such as Vidin, Montana, Pazardzhik, and Plovdiv, to limit the spread of phytoplasma diseases.

Based on the obtained results, it can be concluded that the absence of AP is likely due to years of research and the measures implemented by state authorities and farmers. On the other hand, the limited number of detected infections should heighten our attention, focusing it on regions with large fruit orchards.

Fruit growing in Bulgaria faces challenges related to climate change, the susceptibility of certain crops to diseases, and the need to adopt modern technologies and practices to increase productivity and improve the quality of produce.

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TRAINING SYSTEMS FOR ORCHARDS HIGH-DENSITY CHERRIES FROM THE REPUBLIC OF MOLDOVA

Valerian BALAN, Inna BÎLICI, Stanislav RUSSU, Vasile ȘARBAN, Igor IVANOV, Corneliu BUZA, Dumitru TALPALARU

Technical University of Moldova, 168 Ștefan cel Mare și Sfânt Blvd, Chișinău, Republic of Moldova

Corresponding author email: valerianbalan@gmail.com

Abstract

The study was conducted between 2010 and 2024 in the central and northern fruit growing area of the Republic of Moldova to evaluate the effect of the cherry (Prunus avium L.) training system on fruit growth, yield and quality, comparing four training systems (Thin spindle; Cup; Kym Green Bush; Improved thin spindle) to five varieties (Early Star, Samba, Black Star, Kordia and Regina) grafted on Gisela 6 rootstocks, with trees planted at a distance of 4x1m and 4x2m. The crowns of the trees, at the age of 6-7 years, formed continuous rows in the direction of the row, covering the soil with the projection of the crown at 61-63.7%, having the lateral area of the crown of 21300-22450 thousand m^2/ha and the volume of the crown of 158 m^3/ha 153858-22450. During the fruiting period the trees have a slower growth rate compared to the growing period. The cumulative yield of the cultivar Samba 8.43-10.88 t/ha and Black Star 7.66-9.84 t/ha, grafted on Gisela 6, during the period of growth and fruiting of the trees was higher for the cherries formed according to the thin spindle crown.

Key words*: sweet cherry cultivar, crown, density, productivity, quality.*

INTRODUCTION

Cherry can be grown in different orchard systems and crown shapes, depending on the soil, light, heat, rainfall, etc., the workforce, the available technology and the fruit production market (Babuc, 2012; Balan et al., 2001; Ivanov, 2012). Management and pruning systems have a primary role in cherry cultivation because the crown structure should facilitate harvesting, as well as tree management operations during vegetation (Asanică, 2012; Balan, 2015; Balan and Ivanov, 2016). Tree management forms can be classified into: improved natural, improved modified natural, artificial with various geometric shapes and internal structures. Currently, in the semi-intensive system, the crown forms used are Spanish Bush, Improved Pot, Naturally Improved Palmeta and in the intensive and super-intensive system - Super Fuss Axis, Thin Spindle, Upright Fruiting Offshoots System (UFO) Super Slender Axe (SSA), Tall Spindle Axe (TSA), Drapeau Marchand, Tall Fusiform Axis (Babuc et al., 2015).

Low-volume crown forms such as Improved Pot, Drapeau Marchand, Spanish Shrub, Naturally Improved Palmeta, Kym Green Bush in combination with medium-vigorous rootstocks (Gisela 12, Krymsk 5, Maxma 14, Piku 1, Piku 4, Ceravium ® PHL A) are grown on fertile and less favorable soils (Babuc et al., 2015; Cimpoieș, 2018). Spindle crown shapes such as Super Fuss Axis, Tall Fusiform Axis, Thin Spindle, Super Slender Axe (SSA), Tall Spindle Axe (TSA), in combination with medium-low (Gisela 6, Krymsk 6, P-HL-C) and medium (Gisela 12, Krymsk, 145, Max, 14, 14). The rootstock of vigor Ceravium ® PHL A) is practiced on fertile and irrigated soils from high-density orchards (Balan et al., 2023, 2024; Balmer, Blanke, 2005). Regardless of the shape of the crown, the maintenance pruning of the crown is used according to the physiological state of the tree to obtain the projected shape. In the first 2-3 years after planting, severe pruning intensifies vegetative growth and delays fruiting. In modern orchards, trees are allowed to grow as freely as possible, with no pruning or minimal pruning required (Blažková, Drahošová, 2012; Elfving, Visser, 2009; Ghena et al., 2004; Агафонов, 1983).

Currently, free-growing spindle crown forms such as Tall Fusiform Axis, Tall Spindle Axe (TSA), Super Slender Axe (SSA), Super Fuss Axis, Thin Spindle, associated with vegetative rootstocks of different vigor in plantations with

high density (2500-3000 trees/ha) are accepted. These crowns allow the formation of continuous, well-ventilated and illuminated rows, the filling of branches with fruit formations, the early entry into fruiting and obtaining large harvests, the making of pruning and harvesting from the ground or from small ladders (Babuc, 2012; Long et al., 2014). Modern fruit growing provides for minimizing pruning during the growing period of trees and replacing them with horizontal routing of shoots to slow down growth and differentiate buds from fruit earlier, as well as early fruiting of trees. Early harvests attenuate the vegetative growth characteristic of this stage. Throughout the fruiting period, tree pruning is carried out by maintenance pruning and fruiting to ensure harvests year after year (Balan et al., 2001; Bennewitz et al., 2011; Cimpoieș, 2000).

The purpose of this research was to evaluate the effect of the cherry formation system on the growth, yield and quality of cherry fruits (Prunus avium L.) in the central and northern areas of the Republic of Moldova. Theoretical and experimental studies focused on six crown shapes: Naturally improved crown with low volume (control); Thin spindle; Cup; Kym Green Bush; Improved thin spindle.

MATERIALS AND METHODS

The study was carried out between 2010 and 2024 in the central and northern fruit growing area of the Republic of Moldova at Ustia LLC "Staragrogroup" Dubasari district and ProdCar LLC, Negureni commune, Telenesti district and in the cherry orchard of the Sermofarm company, Sturzeni village, Riscani district.

Experience 1 was established in the autumn of 2015 with the Early Star, Samba, Black Star cherry varieties, grafted on the Gisela 6 rootstock, with the trees planted at a distance of 4x2 m. Low-volume crown shapes have been studied: Thin spindle; Cup; Kym Green Bush.

Experience 2 was organized in Sturzeni, in the spring of 2018 with the Kordia and Regina varieties, grafted on Gisela 6, at a distance of 4 x 1 m. Trees are led by the classic slender spindle crown and improved thin spindle.

In all orchards, the varieties are grouped in bands of 8-10 rows of each cultivar, with the rows of trees oriented from north to south. The formation of the crown and the vigor of the trees were controlled by the method of double sectoral pruning (Babuc, 2012) and the cutting of branches that exceed half of the growth vigor of the branches on which they are located, with the aim of optimizing the growth ratio of the vegetative and reproductive organs in order to accelerate the entry of the trees into fruiting. The half-skeleton branches are periodically renewed by rotation once every 3-4 years.

The researches were carried out, in terms of tree formation and pruning, according to the methodical guidelines for carrying out investigations with fruit species (Moiseicenco et al., 1998). To determine the influence of the crown formation system of cherry trees on the growth, fruiting and productivity of trees, the following were performed: biometric analyses (trunk diameter, crown height and width, average and sum length of annual branches, yield, fruit mass and diameter); biochemical analyses (dry matter, total sugar content, titratable acidity, fruit fermity) (Balan et al., 2001).

The morphological studies of the trees were carried out annually, at the end of the growing season. At 32 trees, the height of the trees and the width of the crown were measured, at 4 trees, the number of vegetative branches, the average and total length of the annual branches was determined.

The harvest was determined at the maturity stage of fruit consumption, for each tree separately by weighing the fruits from 32 trees in the variant and calculating the arithmetic average, and the yield was reported per hectare. During the fruit development and ripening period, the diameter and mass of the cherries were determined using a template (VOEN, Germany) with holes of 24, 26, 28, 30, 32, 34 and 36 mm, corresponding to a mass of 7, 8.5, 10, 11.5, 13, 14.5 and 16 g, respectively. These analyses were recorded on 20 cherries in four identical samples (n=80) of each variety. The average weight of the cherries was determined at the time of harvest by weighing a 1 kg sample of cherries using a digital scale (model AS 82/220.X2) with an accuracy of ± 0.01 g, and by counting the cherries in each sample.

The soluble substance content in the fruit was measured directly in the orchard using a digital refractometer (models ATAGO N-20E and

DR201-95), with results expressed as a percentage of Brix.

Titration acidity was established by neutralization with 0.1 N NaOH solution in the presence of phenolphthalein, expressed as malic acid in %. Fruit hardness was measured using an AGROSTA 100 penetrometer produced by Firm Tech with a favorable measurement value for cherry fruits above 250 g/mm^2 (Long et al., 2014). For the interpretation of the scientific results, methods such as analysis, synthesis, tabulation, comparison, and graphing were employed. The data were processed and presented as average values over the research years.

The study results were verified through analysis of variance (ANOVA) using Microsoft Office Excel 2003. Differences between the treatment options were compared at a significance level of 0.05 using the Tukey test (Dospëkhov, 1985).

The experimental plots are characterized by flat to slightly fragmented terrain, fertile soils, moderate water availability, and weak erosion processes. Predominant strong winds come from the north, and the frost-free period ranges from 180 to 190 days. The first frosts are visible from mid-October, the last frosts in mid-May. In recent decades, the average air temperature in the region was 11.2-11.9°C, and during the vegetation period it was 16.8-17.5°C. The average annual duration of sunshine is 1950 hours in the north and 2210 hours in the south, which is 50-55% of the possible duration. The average annual air temperature in the country, according to multiannual data, was 10.9°C, and during the vegetation period - 17.3°C (Balan et al., 2021). The duration of the vegetation period of 180-185 days with temperatures above 10°C is about 2200-2300 hours annually, and the frost-free period is about 280-290 days.

Agrotechnical measures shall be carried out in accordance with the agrotechnical guidelines in force. The orchards are irrigated by dripping, and Watermark transducers installed at a depth of 20, 40 and 60 cm in each plot are used to monitor soil moisture. The water is distributed through the network with drippers fixed 40 cm from the ground in the direction of the row. The soil in the orchards in the first two years, after planting the trees, was maintained as worked land, but in the following years the distance between the rows is maintained with artificial grass. The strips between the rows, 2 - 2.5 m wide, with weeds that grow naturally and artificially, are pruned as needed and remain as mulch. Herbicides or 2-3 mechanical pruning are applied along the row of trees, with a probe cutter. Soil maintenance, irrigation, fertilization and protection of trees from diseases and pests are carried out as appropriate (Babuc, 2012).

RESULTS AND DISCUSSIONS

Modern fruit growing requires crown shapes that maximize the conversion of solar energy into fruit, as well as labor productivity during manual pruning and harvesting. These considerations required the research approach of a large number of cherry crowns under different ecological conditions.

Tree growth. The height of the cherry trees differs depending on the shape of the crown (Table 1).

Table 1. Height and length of the crown of cherry trees depending on the variety and crown shape, cm
(Gisela rootstock 6, planting distance 4 x 2 m, age of the trees 4-7 years)

Crown shape	Tree height, cm				Crown length, cm			
	2018	2019	2020	2021	2018	2019	2020	2021
Early Star variety								
Thin spindle	377.0	378.3	369.6	385.0	125	175	211	240
Cup	340.3	342.6	333.3	348.7	144	192	220	251
Kym Green Bush	318.3	316.0	295.0	319.7	150	200	232	240
Samba variety								
Thin spindle	385.0	400.0	400.0	375.0	134	185	200	250
Cup	362,6	346.3	351.6	363.5	146	176	234	245
Kym Green Bush	321.6	300.0	315.0	312.2	145	195	230	250
Black Star variety								
Thin spindle	378.3	392.3	392.0	397.5	145	214	215	245
Cup	329.0	355.0	348.3	354.1	162	222	200	250
Kym Green Bush	307.0	306.3	312.0	318.4	138	195	229	250
DL 5%	53.2	42.9	39.8	62.7	-	-	-	

The shape of the thin spindle crown is highlighted by a higher height of the tree, compared to the cup shape and Kym Green Bush. The Early Star variety, in the 4th year after planting, recorded a tree height of 318.3-377.0 cm, being higher in the case of the thin spindle crown. In the following years 2019-2021, the increase in height was similar to that of 2018, in the sense that the most vigorous trees were recorded in the crown of the thin spindle. The Kym Green Bush system forms lower crowns compared to the cup shape and the thin spindle. The Samba variety of medium to high vigor, in the years of study, has formed trees with a height of 300-321.6 cm in the crown shape Kym Green Bush up to 375-400 cm in the shape of a thin spindle crown, and the crown shape cup occupies an intermediate position (346.3-363.5 cm).
The height increases of the trees of the Black Star variety are similar to the trees of the Early Star and Samba varieties in that the smallest trees were in the case of trees formed according to the Kym Green Bush system (306.3-318.4 cm). Thus, the height of the Early Star, Samba and Black Star cherries, grafted on the Gisela 6 rootstock, was significantly higher in the trees formed after the thin spindle crown compared to the Kym Green Bush system and was not always significantly ensured by the crown shape of the cup. The width of the crown of cherry trees depends on the shape of the crown and the age of the trees (Figure 1).



Figure 1. Crown width of cherry trees of the Samba variety according to the crown shape, cm (Gisela rootstock 6, planting distance 4 x 2 m, age of trees 4-7 years)

The trees at the age of 4, in the Samba variety, had a crown width from 150 cm in the shape of the crown of the cup to 166 cm in the case of the Kym Green Bush system. As the trees aged, the width of the crown also increased, reaching maximum sizes in years 6-7 after planting. Thus, at the age of 6 the width of the crown was 230-236 cm and 246-253 cm at the age of 7, being larger and larger in the form of the Kym Green Bush. The Early Star and Black Star varieties manifested themselves identically to the Samba variety, forming a crown of optimal size in the 6-7 years after planting in the orchard. The width of the crown depends on the distance between the rows and the height of the trees. The width in the basal part of the crown, in the Early Star, Samba, Black Star cherry varieties, grafted on the Gisela 6 rootstock, planted at a distance of 4 x 2 m, was maintained at about 250 cm, regardless of the shape of the crown.
The morphological parameters of the crown allowed to calculate the lateral area and volume of the crown, as well as the level of ground cover with the projection of the crown, (Table 2).
The indicators of the vegetative ensemble of the orchard are phytometric parameters that determine the productivity of the plantation from a theoretical and practical point of view, including the use of solar energy in the photosynthesis process, as well as the management of orchard maintenance (Balan et al., 2018; Jackson, 1980; Odier, 1978). Early Star cherries at the age of 4 years use over 50% of the area reserved for planting, and at the age of 7 years the soil cover with crown projection has increased to 61-63.7%, being higher in the case of the Kym Green Bush training system. At the age of 4 years, the lateral area of the crown is 20850-21900 thousand m^2/ha and 21300-22450 thousand m^2/ha at the age of 7 years. The volume of the crown, being an indicator of the synthesis of the dimensional parameters of the vegetative ensemble of the plantation, was from 13666-18881 m^3/ha in 2018 and increased to 15688-20535 m^3/ha in 2021. Trees formed without a central shaft, according to the crown cup and Kym Green Bush formed a higher volume by 29.1-38.2% in 2018 and by 18.9-30.9% in 2021, compared to the thin spindle crown shape. a tree height of 318.3-377.0 cm, being higher in the case of the thin spindle crown. In the following years 2019-2021, the increase in height was similar to that of 2018, in the sense that the most vigorous trees were recorded in the crown of the thin spindle.

Table 2. Structure of the vegetative ensemble of cherry trees, of the Early Star variety depending on age and crown shape (Gisela rootstock 6, planting distance 4 x 2 m, age of trees 4-7 years)

Crown shape	Crown height, cm	Crown width, cm		Land cover level, %	Crown side area, m^2/ha	Crown volume, m^3	
		At the base	at the top			tree	Ha
Year 2018, age of trees – 4 years							
Thin spindle	377	210	80	52.5	20850	10.9	13666
Cup	340	220	195	55	21875	14.1	17637
Kym Green Bush	318	235	240	58.7	21900	15.1	18881
Year 2021, age of trees – 7 years							
Thin spindle	385	244	82	61	21300	12.5	15688
Cup	348	243	186	60.7	22050	14.9	18661
Kym Green Bush	319	255	260	63.7	22450	16.4	20535

The morphological parameters of the structure of the vegetative ensemble in cherry trees of the Samba and Black Star varieties also formed the highest values of the lateral surface, the volume of the crown and the level of soil cover with the projection of the crown, were in the trees formed according to the crown of the cup and Kym Green Bush.

In conclusion, we note that the geometric structure of the vegetative ensemble of the fruit plantation of the Early Star, Samba, Black Star varieties, grafted on the Gisela 6 rootstock, planted at a distance of 4 x 2 m, ensures an optimal structure for the penetration of solar energy into the center of the crown in the roofs and sub-roofs, which are garnished with the largest possible volume of productive branches. The above-mentioned varieties form a continuous crown volume in the row direction, which at the age of 4 years receives more than 50% of solar energy, and at the age of 7 - over 60%.

The growth of the annual branches is determined by the variety, the age of the trees and the shape of the crown (Figure 2).

During the growing period of the trees, the length of the annual branches, by years, was constantly decreasing. For example, in the Samba variety, the average length of the annual branches was 75.2-80.4 cm in the 4th year after planting, 61.4-71.7 cm in the 5th year, 35.8-42.7 cm in the 6th year and only 32.5-34.8 cm in the 7th year after planting. The shape of the Kym Green Bush crown recorded higher annual growth values compared to the thin spindle and cup crown, but they are not always statistically assured. The annual growth of the Early Star and Black Star varieties recorded values similar to the Samba variety, in the sense that as the trees age, the length of the annual branches decreases.

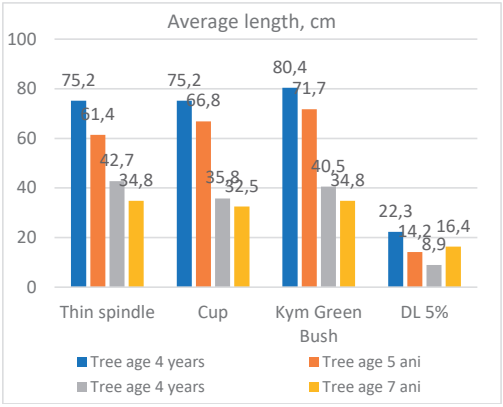


Figure 2. Average length of annual branches of cherry trees of the Samba variety according to crown shape, cm (Gisela rootstock 6, planting distance 4 x 2 m, age of trees 4-7 years)

The total length of annual cherry branches differs from variety to variety, from the age of the trees and from one crown shape to another (Figure 3).

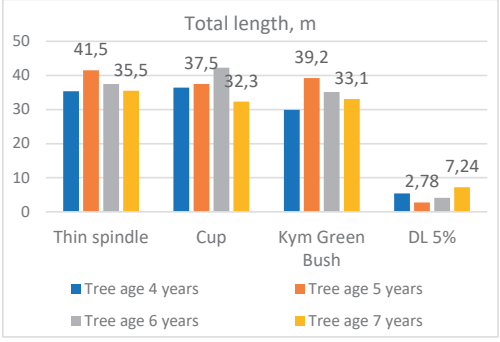


Figure 3. The sum length of the annual branches of the cherry trees of the Samba variety according to the crown shape, m (Gisela rootstock 6, planting distance 4 x 2 m, age of the trees 4-7 years)

Thus, at the beginning of the growth and fruiting period of the trees, the total length of the annual branches increases, and towards the end of this period the growth on the tree attenuates to the optimal level in order to obtain constant harvests of quality fruits. In addition, a balance is established between growth and fruiting (Babuc, 2012; Wertheim, 1997).

Fruit yield. During the period of tree growth and fruiting, the average cherry fruit harvest, in the first 7 years of fruiting, demonstrates that the Early Star variety had a lower yield compared to the Samba and Black Star varieties. This decrease in yield in the Early Star variety is due to the fact that the variety with the earlier fruit ripening period is in more unfavorable conditions (rain, frost) for the development of reproductive organs (flowers, fruits).

If we refer to the influence of the shape of the crown, it can be seen that the thin spindle crown during the period of growth and fruiting of trees has advantages compared to cup-shaped crowns and Kym Green Bush. For example, in the Samba variety, fruit production (8.70 t/ha) in most years was significantly higher compared to cup-shaped crowns and Kym Green Bush. The same pattern was recorded for the Early Star and Samba varieties, in the sense that the highest distinctly significant values with 11.8-18.4% (cup) and 20-29% (Kym Green Bush) were for trees formed after the thin spindle crown.

Orchard productivity is the basis for calculating the efficiency of tree cultivation technology, including variety-rootstock association, planting distance, crown shape, tree pruning period and method, as well as orchard management (Balan et al., 2021; Budan, Amzar, 1992; Ivanov, 2023; Ivanov et al., 2018; Pește anu et al., 2019; Stehr, 2008).

Thus, cultivation systems are obtained that are suitable for the conversion of solar energy with a high coefficient by trees. For these reasons, the success of an orchard is appreciated by its precocity, constant yield and competitive fruit quality on the market (Babuc, 2012; Ivanov et al., 2023).

The Early Star and Samba cherry trees, grafted on the Gisela 6 rootstock, planted at a distance of 4 x 2 m, began to bear fruit in the 4th year after planting with a yield of 2.31- 3.67 t/ha (Table 3).

Table 3. Fruit harvest of cherry trees by variety and crown shape, t/ha (Rootstock Gisela 6, planting distance 4 x 2 m, age of trees 4-10 years, SRL "StarAgroGroop")

Crown shape	Year 2018	Year 2019	Year 2020	Year 2021	Year 2022	Year 2023	Year 2024	Intercede (2018-2024)
Early Star variety								
Thin spindle	2.94	7.17	4.87	9.45	11.01	3.64	4.01	6.16
Cup	2.31	6.40	4.25	8.27	9.41	4.06	3.81	5.50
Kym Green Bush	2.56	5.35	3.54	7.67	9.51	3.84	3.49	5.14
DL 5%	-	1.23	0.56	0.87	0.62	1.24	1.46	-
Samba Variety								
Thin spindle	3.67	16.97	4.08	16.26	9.89	8.16	17.15	10.88
Cup	2.80	14.53	3.58	12.88	8.11	6.84	15.51	9.19
Kym Green Bush	2.19	12.96	4.04	10.30	8.27	7.21	14.05	8.43
DL 5%	-	1.34	1.1	1.72	1.16	0.28	1.15	-
Black Star variety								
Thin spindle	0	13.01	10.29	5.50	9.39	9.96	10.92	9.84
Cup	0	12.01	9.45	3.73	8.81	8.11	9.67	8.63
Kym Green Bush	0	10.62	7.66	3.90	7.96	7.65	8.22	7.66
DL 5%	-	2.13	1.41	0.39	0.28	1.17	1.34	-

The data presented show that the Early Star variety has had a modest fruit yield over the years. Over the years, the spindle crown formation system has recorded the highest fruit yields (2.94-11.01 t/ha) compared to the cup crown forms (2.31-9.41 t/ha) and Kym Green Bush (2.56-9.51 t/ha), but they have not always been significantly ensured. The yield of the Early Star variety, with fruit ripening in the first half of June, was 5.14-6.16 t/ha on average over 7 years of fruiting. The Samba variety, with the fruit ripening period in the second decade of June, was more productive compared to the Early Star variety. Thus, the trees formed after

the thin-spindle crown had a yield of 3.67-9.89 t/ha, and the harvest was clearly significant compared to the Kym Green Bush system, but the difference was not always statistically ensured compared to the shape of the cup.

The Black Star variety, self-fertile, with an average fruit ripening period, entered fruiting in the 5th year after planting, with a yield of 7.66-9.84 t/ha, on average over the first 6 years of fruiting. In this variety, the highest harvest values, distinctly significant, were the trees formed according to the shape of the thin spindle crown. For example, in 2020 the yield of the Black Star variety was 7.66-10.29 t/ha, being 7.6% higher in the case of the cup shape and 34% higher than the Kym Green Bush system.

Effects of fruit quality parameters. The quality of the fruits is directly related to the maintenance, formation and pruning of the crown as well as to the cultural management applied in the orchard (Balan, Ivanov, 2014; Balan et al., 2021; Blažková, Drahošová, 2012; Manziuc, Fedorciucov, 2021). Regardless of the cropping system, fruit quality requires cherries to be picked at the right time, firm, uniform in color, and resistant to cracking, crushing, and hollowing out (Balan et al., 2017).

Table 4. Influence of the variety on the quality of fruits in cherry trees (Gisela rootstock 6, planting distance 4 x 2 m, thin spindle crown, tree age 6 years, year 2021)

Variety	Fruit diameter, mm	Cherry mass, g	Fruit firmness, kg/cm^2	Soluble dry matter, %	Titratable acidity, mg malic acid 100 g^{-1}
Early Star	28.58	10.71	2.79	17.4	0.71
Samba	30.23	11.92	3.04	18.5	0.69
Blak Star	30.48	12.05	2.89	19.2	0.72
DL 5%	0.53	0.75	-	-	-

The diameter of cherries is a morphological indicator of quality and serves as a basis in the technological production process, including the modification of post-harvest fruit processes such as packaging, sorting and marketing of cherries (Ivanov et al., 2015; Long et al., 2014; Mitre et al., 2012; Mong et al., 2004; Pește anu et al., 2018; Șarban, 2021). The size of the fruit differs from the care of the orchard and the climatic conditions, being significantly higher in the Samba and Black Star varieties, compared to the Early Star variety (Table 4). The cherries of the studied varieties are of high quality, especially in the Black Star variety, in which the diameter was 30.48 mm with a mass of 12.5 g. The soluble dry matter in the fruit was 17.4-19.2%, titratable acidity - 0.69-0.72 mg malic acid 100 g^{-1} and 2.79-3.04 kg/cm^2 fruit firmness. It should be noted that the quality parameters of the Early Star, Samba and Blak Star varieties, grafted onto the Gisela 6 rootstock, are variety-specific values and differ slightly from the climatic conditions.

Cherry trees in high-density orchards, due to the strong apical dominance and the natural tiering of branches, are stripped in the basal part of the crown (Balan et al., 2001). The improved thin spindle is used in high-density plantations, in various tree formation combinations. The process of forming cherries in an improved thin spindle form includes a well-developed axis, at the base of which, between 60-100 cm in height, 4-6 permanent semi-skeletal branches are selected, radially around the axis, followed by a long zone of 90-100 cm free of branches, then the axis is trimmed with weak semi-skeletal and fruit branches (Balan et al., 2024). This procedure allows the formation of conical crowns, with a well-developed central axis and covered with semi-skeletal branches and fruit branches, which decrease in size from the base to the top of the tree and ensure optimal use of solar energy to increase the size and quality of the fruits.

The preliminary results of the improved thin-spindle crown form are promising (Table 5). The yield of trees of the Regina (4.56-4.76 kg/tree) and Summit (6.63-6.91 kg/tree) varieties, grafted on Gisela 6, does not differ from the crown formation system, however, the diameter of fruits of 30 mm and more is greater in the case of tree formation according to the improved

thin-spindle system. For example, in the Summit variety formed after the improved Thin-stem crown, fruits with a diameter of 22-26 mm and 26-30 mm decreased by 10.08% and 9.53% respectively, but fruits with a diameter of 30 mm and more increased by 19.6%. The same pattern was also recorded in the Regina variety.

Table 5. Distribution of cherry fruits by variety and crown shape
(Gisela rootstock 6, planting distance 4 x 1 m, 7 years)

Crown formation system	Yield, kg/tree	Fruit diameter, mm		
		22-26	26-30	> 30
		Fruit weight, %		
Regina variety				
Thin spindle	4.76	23.53	50.03	26.43
Thin spindle improved	4.56	15.03	46.82	38.16
Summit variety				
Thin spindle	6.91	25.21	51.06	23.73
Thin spindle improved	6.63	15.13	41.53	43.33

CONCLUSIONS

The research, carried out between 2010 and 2024, aimed to increase the productivity of cherry plantations by identifying a cherry tree training system to obtain efficient competitive fruit crops and maintain a balance between growth and fruiting.

The crowns of the Early Star, Samba, Black Star varieties, at the age of 6-7 years, formed continuous rows in the direction of the row with a width of about 250 cm, regardless of the shape of the crown. The level of soil cover with crown projection at the age of 7 years increased to 61-63.7%, the lateral area of the crown was 21300-22450 thousand m^2/ha, and the crown volume increased to 15688-20535 m^3/ha in 2021.

The annual growth of Early Star, Samba, Black Star varieties recorded higher values (75.4-92.4 cm) during the tree growth period and decreased (45.2-49.5 cm) during the fruiting period when a balance between growth and fruiting is established (42.2-48.2 cm).

The yield of the trees grafted on Gisela 6, during the growth and fruiting period, was 5.14-6.16 t/ha for the Early Star variety, 8.43-10.88 t/ha for the Samba variety and 7.66- 9.84 t/ha for Black Star, being higher for the cherries formed according to the thin spindle crown.

The process of forming cherries into an improved thin spindle shape (Balan et al., 2024) should be further analyzed to identify methods to maintain trees in physiological balance between growth and fruiting, during the period of full fruiting.

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THE INFLUENCE OF PRUNING ON VEGETATIVE GROWTH, YIELD AND QUALITY OF CHERRY FRUITS OF THE 'KORDIA' VARIETY GRAFTED ON 'MAXMA 14' (*PRUNUS AVIUM*)

Inna BÎLICI, Valerian BALAN, Vasile ȘARBAN, Corneliu BUZA, Dumitru TALPALARU

Technical University of Moldova, 168 Ștefan cel Mare şi Sfânt Blvd, Chişinău, Republic of Moldova

Corresponding author email: inna.bilici@h.utm.md

Abstract

The paper refers to the influence of tree pruning on vegetative growth, harvest and fruit quality of the Kordia cherry cultivar grafted onto the MaxMa 14 rootstock. Investigations at SRL "StarAgro Group" (Ustia village, Criuleni district) were organized in the central fruit-growing area of the Republic of Moldova, with the Kordia cultivar, grafted onto the Maxima 14 rootstock. The vegetative growth of the trees in the cherry variety under study is expressed, in quantitative terms, by the volume of vegetative growth accumulated annually through the height and width of the tree crown, through the size of the surface and volume of the crown, as well as the level of soil coverage by the vegetative ensemble of the trees. The cherry variety, Kordia, grafted on the MaxMa 14 rootstock, had yields from 18.10 kg/tree where pruning was performed during the dormant period, to 19.78 kg/tree in the variant where pruning was performed in early autumn.

Key words*: cultivar, rootstock, harvest.*

INTRODUCTION

Cherry cultivation is among the most attractive fruit crops because cherries are fruits (consumed fresh) appreciated, preferred and sought after by consumers, and the selling price is almost always high (Ivanov I. and Balan V., 2017).

The total area of the world occupied by cherries is over 440 thousand ha, and the global production of cherries is about 2.3 million tons per year, of which 35% comes from Europe. Given the favorable ecological growing conditions, in the Republic of Moldova cherries occupy approximately 4100 ha, with a fruit production of over 10 thousand tons annually, but with a fairly modest yield, below the world average (Cimpoieș Gh., 2018).

Among fruit crops, cherry has experienced the highest evolution in the last 20 years due to the new assortment of self-fertile, high-quality varieties, and the cultivar of vegetative rootstocks of low and medium vigor.

The multiple existing cultivar - rootstock associations, the numerous planting distances, as well as the diversity of biological material have required an increase in the number of researches on tree management systems (Long L. E. et al., 2014).

The pressing problems faced by fruit growers in cherry production are the efficient use of natural resources such as soil, light, slope and exposure of the land, etc. Therefore, it is appropriate to identify the biotic and abiotic factors that define the crop system, which corresponds to the biological production potential of the orchard and economic interests. Obviously, the following principles will be at the heart of the design of the orchard system: geographical conditions and the degree of natural fertility of the soil, the relative vigor of the cultivar-rootstock association, planting density, early and high yields; simple crowns easily adaptable to partial mechanization. At the moment, the cherry crop has varieties and rootstocks of different vigor, admits different forms of crown management, offers the possibility of using all crop systems, accepts the adaptation of the orchard to less fertile soils and sloping lands. (Balan V. et al., 2021).

In modern fruit growing, the cherry cultivation system provides for the introduction of rootstocks of low (Gisela 5), medium-low (Gisela 6, P-HL-C, Krymsk 6) and medium (Gisela 12, Krymsk 5, MaxMa 14, Piku 1, Piku 4) vigor, simple crown shapes, which fully utilize the nutritional space offered to the tree,

early entry of trees into fruiting, high quality and efficient harvests, mechanization of technological processes and increased labor productivity in maintenance and harvesting. At the same time, it is necessary to analyze not only biological and technological factors but also social and economic ones such as labor force, tree protection (frost, hail, rain, etc.), the use of fertilizers, irrigation and pesticides, mechanization of pruning and fruit thinning. Peșteanu A. and Șarban V., 2022).

Current scientific research shows that cherries have the role not only of satisfying the consumer with fresh fruit, but also have an opportunity as a raw material for the food industry, and due to their nutritional and therapeutic content, they are increasingly in demand on the market at a captivating price for producers. Cherry is cultivated everywhere and capitalizes on lands with varied slopes and soils, ensuring harvests every year (Peșteanu A. and Șarban V., 2022).

The improvement of pruning technology by introducing new methods obtained worldwide induces pressures on already existing cultivation technologies, and the latter must be shaped to maximize the superior biological potential of the new creations. One of the technological links that has successfully managed to maintain a reduced tree size is maintenance and fruiting pruning (Cimpoieş, 2000; Stefanco et al., 2009).

The purpose of this work is to evaluate the productivity and quality of cherry fruits of the Kordia variety depending on the types of pruning.

MATERIALS AND METHODS

Investigations at the LLC "StarAgro Group" (Ustia village, Criuleni district) were organized in the central fruit-growing area of the Republic of Moldova, with the Kordia cultivar, grafted onto the MaxMa 14 rootstock. The trees were planted in the fall of 2012 at a distance of 5 x 3 m, using the improved natural crown shape with reduced volume. Maintenance and fruiting pruning of cherry trees was carried out during the dormant period and the growing season according to the following variants: V1 - Pruning during the dormant period (control); V2 - Pruning during flowering; V3 - Pruning after harvest (July); V4 - Pruning in early autumn (first decade, September).

The experiment was organized in 4 repetitions of 8 representative trees in each.

Agrotechnical measures in the orchard are carried out in accordance with the recommendations in force. In the LLC "StarAgro Group" orchard, drip irrigation is used, and to monitor soil moisture, Watermark transducers are used, installed at 20, 40 and 60 cm depth in each plot. Water is distributed through mains with drippers fixed 40 cm from the ground in the direction of the row.

In experiments, in the first two years after planting the trees, the soil was maintained as a cultivated field, in the following years the distance between the rows remained grassed naturally or artificially. The strips between the rows are mowed as needed and remain as mulch. Herbicides or 2-3 mechanical plows are applied along the row of trees, with a rotary tiller.

RESULTS AND DISCUSSIONS

The vegetative growth of trees in the cherry cultivar under study is expressed, in quantitative terms, by the volume of vegetative growth accumulated annually through the height and width of the tree crown, the surface area and volume of the crown, as well as the level of soil coverage by the vegetative ensemble of the trees (Table 1).

After evaluating the relationships between the parameters of the structure of the fruit plantation, it was demonstrated that in the Kordia cultivar at a row spacing of 5 m, the crown height varies from 370 cm in 8-year-old trees to 400 cm in 11-year-old trees. The crown width at the base recorded maximum values of 230 cm in 8-year-old trees to 240 cm in 11-year-old trees.

The crown width at the top reached values of 92 cm in 8-year-old trees and a greater crown width at the top was recorded in 11-year-old trees.

The level of soil coverage with the vegetative ensemble receiving solar energy has values of 46.1-48.1%.

The lateral crown area varies from 16623 m^2/ha in 8-year-old trees and increased considerably in 2021, in 11-year-old trees it is 17982 m^2/ha.

Table 1. Structure of the vegetative ensemble of cherry trees (Kordia cultivar) depending on age and biological characteristics of the cultivar

Crown height, cm	Crown width, cm		Soil cover level, %	Lateral crown area, m^2/ha	The volume of the crown, m^3	
	at the base	at the top			tree	Ha
Year 2018, age of trees – 8 years						
370	230	92	46,1	16623	17.8	11902
Year 2021, age of trees – 11 years						
400	240	100	48,1	17982	20.4	13586

The crown volume, which depends on the crown area, in 8-year-old trees is 17.8 m3/tree and, respectively, 11902 m^3/ha. In 2021, the crown volume increased compared to 2018, and is 13586 m^3/ha. In the 11th year of vegetation, the Kordia cherry trees achieved an optimal crown area and volume, which characterizes the productive potential of the plantation.

Along with the growth and development of the trees, the crown height also increases. In 8-year-old trees, the height of the trees varies depending on the period of their pruning and is from 370 cm (Table 2) in trees where pruning was performed during the dormant period (control) to 400 cm in trees where pruning was performed in early autumn. In the variants where pruning was carried out during flowering and pruning after harvest, the height of the trees is 390 cm.

Table 2. Height of cherry trees (Kordia cultivar) depending on the period of tree pruning (cm)

Tree pruning period	Years			
	2018	2019	2020	2021
V1	370	380	390	400
V2	390	400	400	400
V3	390	400	400	400
V4	400	400	*395*	*415*

Trees aged 11 years (2021) have reached the optimal height, so in variants (1, 2, 3) where pruning was carried out during the dormant period, during flowering and after harvesting, the height of the trees is 400 cm. The trees reached a greater height in the variant where pruning was carried out in early autumn - 415 cm.

The growth in length of the shoots on the stem varies depending on the species, the cultivar - rootstock association, the age of the trees, the location of the branches in the crown and the agricultural techniques used (Babuc V., 2017). The growth potential is very high during the tree growth period and gradually decreases with age. Physical growth is intense in young trees, slow and prolonged in mature trees. (Cimpoieş Gh., 2020).

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The growth of branches is determined by the cultivar and age of the trees. Thus, in the orchard of SRL "Star Agro Grup", in the 8th year after planting, the average length of annual branches (Table 3) of the Kordia cherry cultivar was from 28.7 cm in the variant where pruning was performed during flowering to 42.7 cm in the variant where pruning was performed after harvesting. In the control variant, where pruning was performed during the dormant period, the average length of annual branches is 34.5 cm. In 9-year-old trees, the average length of annual branches increased compared to the previous year, so in trees where pruning was performed after harvesting this index is 50.6 cm.

Table 3. Average length of annual branches in cherry trees (Kordia cultivar) depending on the tree pruning period, cm

Tree pruning period	Years			
	2018	2019	2020	2021
V1	34.5	41.2	35.6	35.6
V2	28.7	32.5	29.6	30.7
V3	42.7	50.6	45.2	44.2
V4	38.7	42.8	45.3	44.2
DL 5%	3.48	3.74	8.26	4.16

In 2020, in 10-year-old trees, the average length of annual branches reaches higher values in the variants where pruning was performed after harvest (45.2 cm) and pruning in early autumn (45.3 cm).

In 2021, the trees grew equally well and the length of the annual branches reached 30.7-44.2 cm. The Kordia cultivar shows a greater growth of annual branches in the variants where post-harvest pruning and early autumn pruning were performed.

The cherry tree bears fruit on May bunches, medium and long branches. On average across varieties, about 75% of the flowering buds are formed on May bunches, and 25% on medium and long annual branches. On annual branches 30-45 cm long, flowering buds are located predominantly in the lower part. Most of the May bunches with higher productivity potential are located on 2-4-year-old branches.

According to Table 4, in the Kordia cultivar, the number of bouquet branches varies depending on the period of tree pruning but also depending on their location on branches of different ages. In 10-year-old trees, the number of rosettes on 2-year-old wood varies from 89.1 pcs. in the control variant (pruning during the dormant period) to 102.7 pcs. in the variant where pruning was performed during flowering.

Table 4. Number of bunch branches in cherry trees of the Kordia cultivar depending on the tree pruning period, pcs.

Tree pruning period	Rosettes on wood by:			Total, pieces
	2 years	3 years	4 years	
V1	89.1	45.5	3.0	137.6
V2	102.7	48.9	1.8	153.4
V3	99.6	54.9	6.1	160.6
V4	88.0	48.8	2.1	138.9
DL 5%	42.13	15.24	5.54	33.87

The number of bouquet branches on 3-year-old wood varies from 45.5 pcs. in the control variant to 54.9 pcs. in the trees where pruning was performed after harvesting. On 4-year-old wood, the number of rosettes is very small and varies from 1.8 pcs. in the variant with pruning during flowering to 3.0 pcs. in the control variant.

The density of cherry blossoms depends not only on the length of the branches but also on their age. In the Kordia cultivar, it is observed that the largest number of bouquet branches is formed on 2-year-old wood, followed by a smaller number on 3-year-old wood and very few rosettes on 4-year-old wood.

If we analyze this index in total, we can mention that the largest number of rosettes was recorded in variant 3 (cutting after harvest) 160.6 pcs.

The location of the buds in the cherry tree depends on the length of the branch on which they are formed (Table 5). Depending on the length of the annual branches, their diameter also differs, analyzing this index we can mention that with the increase in the length of the annual branches, their diameter will also increase, thus short branches 20-40 cm register a diameter of 5.8 mm compared to the length of the branches 20-100 cm which reached 12.8 mm in diameter.

Table 5. Distribution of buds in cherry trees of the Kordia cultivar depending on the length of annual branches, pcs.

Length of annual branches, cm	Diameter of annual branches, mm	Total number of buds, pcs.	Number of flowering buds, pcs.
20-40	5.8	17.8	7.7
20-60	7.4	21.4	5.4
20-80	9.8	28.0	4.6
20-100	12.8	36.0	3.4

The same pattern is observed if we analyze the total number of buds along the length of the annual branches, their number will increase from 17.8 pcs. on branches with a length of 20-40 cm to 36.0 pcs. on branches with a length of 20-100 cm.

In the 10th year of vegetation, 7.7 pcs. of flowering buds are found on short branches of 20-40 cm, 5.4 pcs. on branches of 20-60 cm, 4.6 pcs. on branches of 20-80 cm and 3.4 pcs. on branches of 20-100 cm.

It was found that the density of flowering buds is higher on short branches (20 cm) and decreases on medium (40 cm) and long (80-100 cm) branches.

Under favorable conditions for the development of photosynthetic processes and depending on the applied technology, high fruit yields can be obtained, which, in economic terms, would satisfy fruit producers and interest in the crop.

From the values presented (Table 6) regarding the fruit harvest, it results that the Kordia cultivar grafted on the MaxMa 14 rootstock, in the 7th year of vegetation, had a yield of 16.43 kg/tree in all the variants studied. In the 8th year of vegetation, the harvest practically doubled and is from 27.87 kg/tree in the variant where pruning was carried out during flowering to 32.13 kg/tree in the variant where pruning was carried out in early autumn. In 2020, the harvest decreased considerably in all the variants studied (9.61-10.86 kg/tree). In the following year (2021), in trees aged 11, the harvest practically tripled and recorded values of 27.47 (V1) - 32.03 (V4) kg/tree.

Table 6. Fruit yield of Kordia cherry trees depending on the period of tree pruning, kg/tree

Tree pruning period	Years							Mediate (2018-2024)
	2018	2019	2020	2021	2022	2023	2024	
V1	16.43	30.67	10.34	27.47	13.0	14.5	14.3	18.10
V2	16.43	27.87	9.81	29.27	13.7	13.2	14.3	17.79
V3	16.43	30.67	9.61	29.73	14.3	14.1	15.5	18.62
V4	16.43	32.13	10.86	32.03	15.7	15.4	15.9	19.78
DL 5%	-	0.74	0.69	2.33	1.76	2.47	1.04	-

In 2022, the harvest decreased and ranges from 13.0 kg/tree in the variant where pruning was performed in early autumn to 15.4 kg/tree in the variant where pruning was performed in early autumn. In the 12th year after planting, the fruit harvest of the Kordia cultivar reaches values of 13.2-15.4 kg per tree. Approximately the same harvest is recorded in the following year (2024) with the maximum harvest being 15.9 kg/tree in variant 4 (pruning in early autumn).

The cherry cultivar, Kordia, grafted on the MaxMa 14 rootstock, had yields from 18.10 kg/tree where pruning was performed during the dormant period, to 19.78 kg/tree in the variant where pruning was performed in early autumn.

Vegetative growth in trees is determined by biological factors (varieties, rootstocks, resistance to diseases and pests) and technological factors (fruit load, food and water supply), which condition the development of physiological processes (Balan V., Șarban V., Ivanov I., 2017).

CONCLUSIONS

The vegetative growth of trees in the cherry cultivar under study is expressed, in quantitative terms, by the volume of vegetative growth accumulated annually by the size of the height and width of the tree crown, by the size of the surface and volume of the crown, as well as the level of soil coverage by the vegetative ensemble of trees

Following the evaluation of the relationships between the parameters of the structure of the fruit plantation, it was demonstrated that in the Kordia cultivar at a row spacing of 5 m the crown height varies from 370 cm in 8-year-old trees to 400 cm in 11-year-old stands.

Along with the growth and development of the trees, the height of the crown also increases. In 8-year-old trees, the height of the trees varies depending on the period of their pruning and is from 370 cm in trees where pruning was performed during the dormant period (control) to 400 cm in trees where pruning was performed in early autumn. In the variants where pruning was performed during flowering and pruning after harvest, the height of the trees is 390 cm.

Cherry fruit on May bunches, medium and long branches. The density of May bunches in cherry depends not only on the length of the branches but also on their age. In the Kordia cultivar, it is observed that the largest number of bunch branches is formed on 2-year-old wood, followed by a smaller number on 3-year-old wood and very few rosettes on 4-year-old wood.

Under favorable conditions for the development of photosynthetic processes and depending on the technology applied, high fruit yields can be obtained, which, in economic terms, would satisfy fruit producers and interest in the crop. The cherry cultivar, Kordia, grafted on the Maxima 14 rootstock, had yields from 18.10 kg/tree where pruning was performed during the dormant period, up to 19.78 kg/tree in the variant where pruning was performed in early autumn.

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INFLUENCE OF CULTIVAR, ROOTSTOCK, AND PLANTING SYSTEM ON PEACH AND NECTARINE YIELD AND FRUIT QUALITY

Alexandru BUCUR[1], Ana Cornelia BUTCARU[2], Cosmin Alexandru MIHAI[1], Florin STĂNICĂ[1, 2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

Productivity in the orchard can be improved by maximizing preharvest factors, including training systems based on compact two-dimensional canopies, high-density trees, and the availability of efficient size-controlling rootstocks. Improved densities have been combined with planar canopies, providing better mechanical and manual pruning and flower thinning, which enhanced yields while optimizing fruit quality and production costs. The study aimed to assess the productive potential of several peach and nectarine cultivars, determining the quantity of produced fruits correlated to flowers. In 2019-2024, two different training systems, Trident (4.0 x 2.0 m - 1,250 trees/ha - 3,750 axis/ha) and Vertical Axis (4.0 x 1.5 m - 1,666 trees/ha - 1,666 axis/ha), have been tested on 14 peach and 16 nectarine cultivars for temperate climate. Three different rootstocks, GF677, Adesoto, and Mirobolan29C, were used for some cultivars.

Key words*: production, training systems, fruit quality, high-density planting, productivity.*

INTRODUCTION

Peaches belong to the Rosaceae family and are highly valued for their nutritional benefits, flavor, and versatility in fresh consumption and processed products. Peach (Prunus persica Batsch L.) and nectarine (Prunus persica var. nucipersica) cultivation plays a significant role in global fruit production, with increasing demand for high-quality fresh fruit driven by consumer preferences and market trends (Abidi, Moreno Sánchez, and Gogorcena Aoiz, 2018). The European Union ranks as the second largest peach grower after China, with an average yearly production of 3,612,000 tons from 2018 to 2020 and a total harvested area of 206,660 hectares in 2019 (Iglesias and Echeverria, 2022). Proper orchard design is crucial for maximizing productivity and fruit quality in peaches (Anthony and Minas, 2021). The success of these crops is strongly influenced by preharvest factors, including cultivar selection, rootstock choice, and orchard management techniques (DeJong et al., 1999). These factors play a crucial role in determining tree vigor, yield efficiency, fruit quality, and overall orchard profitability (Iglesias and Echeverria, 2022; Firde et al., 2019). In modern fruit production, optimizing planting density and training systems is essential for achieving high productivity, resource use efficiency, and maintaining fruit quality standards (Maldera et al., 2021). High-density orchards with two-dimensional canopy structures have gained increasing attention due to their potential to enhance light interception, improve labor efficiency, and facilitate mechanization (Bastías and Corelli-Grappadelli, 2013). Among the most widely studied training systems, Vertical Axis and Trident have been shown to significantly influence tree architecture, fruiting efficiency, and ease of orchard operations (Reig et al., 2018). New training systems were created, mostly by extending the number of axes to two in Bibaum® (Musacchi, 2008), Bi-Axis (Dorigoni et al., 2011), Tatura trellis, Parallel Y, etc. or to three axes in Drilling (Stănică and Platon, 2011; Widmer and Krebs, 2001), Candlestick (Chandelier) (Vercammen, 2011), 3 Leader system (Elkins and DeJong, 2011), Parallel trident, or even four axes in Mikado (Stănică and Platon, 2011; Widmer and Krebs, 2001). The newly developed planting systems were also appropriate for more strong species such as peach (Caruso et al., 1997),

apricot, plum (Meland, 2001). Recently, in Romania several trials were initiated to evaluate new planting systems, canopies designs, cultivars and rootstocks in order to give efficient solutions for new stone fruit orchards (Stănică and Eremia, 2014). The choice of rootstock plays a crucial role in orchard management, influencing both the design and planting density (Anthony and Minas, 2021; Minas, Tanou, and Molassiotis, 2018).Also rootstock selection is another critical determinant of orchard performance, influencing tree vigor, disease resistance, and fruit quality attributes (Pieper et al., 2024). For example, rootstock breeding efforts in Europe have focused on interspecific hybrids as they possess superior traits for tolerance to high pH, drought, salinity, water logging, and fungal diseases (Reig et al., 2020; Reighard, 2000). This study aims to assess the dynamics of several new peach and nectarine cultivars trained under two different canopy systems, focusing on fruit production and quality in relation to crop load, correlated with the total number of flower and the biochemical analysis of fruit composition. The experiment was conducted from 2019 to 2024.

MATERIALS AND METHODS

The study was conducted in the experimental orchard of the Faculty of Horticulture in Bucharest (44°28′18.14″N and 26°4′13.61″E). In the municipality of Bucharest the climate is moderately continental, with an average annual temperature of 10-11°C. In general, winters are cold, with heavy snow, often accompanied by blizzards, and autumns are long and warm, with early springs. The lowest average monthly temperature is recorded in January, with an average value of -3°C. Summer is very hot, in July the average temperature is 23°C, sometimes reaching 35-40°C. The dominant winds, felt in all seasons, are the east (21.2%), followed by the west (16.3%), the northeast (14.2%) and the southwest (11.2%). Precipitation is low, averaging 585 mm per year, but has a higher flow rate in summer: the highest average monthly amounts of precipitation fall in June (about 85 mm), and the lowest in March (15 mm). On average, precipitation falls on the territory of Bucharest on 117 days/year. The soil type in the Experimental Didactic Field of the Faculty of Horticulture in Bucharest is reddish brown soil. The biological material studied was represented by 14 new peach and 16 nectarine cultivars that are not widely cultivated in Romania's plantation, but also native and foreign varieties found in commercial plantations in Romania. Rootstocks used are GF677, Adesoto, and Mirobolan29C. For some cultivars, two different rootstocks were used for the same cultivar. In this study, the productive performance of several peach and nectarine cultivars was evaluated under two training systems: the Trident and the Vertical Axis. For each cultivar and canopy system, a series of quantitative parameters were determined in order to characterize fruit production. These included the average fruit weight (g/fruit), the number of fruits per tree after manual thinning, the individual tree yield (kg/tree), and the extrapolated yield per hectare (t/ha). In addition, the flower-to-fruit ratio was calculated to assess the efficiency of fruit set. Fruits were manually harvested in two stages, corresponding to the commercial maturity phase for each cultivar and training system combination. The consumption maturity was determined using a DA-Meter device (TR Turoni, Italy), which measures the Index of Absorbance Difference (IAD) - a non-destructive indicator of chlorophyll degradation in the fruit skin, providing an objective estimation of ripening stage and optimal harvest time. These measurements were used to compare the influence of the training system and cultivar on overall yield performance and to identify genotypes with superior productive potential under the given orchard conditions. The total number of flowers was determined for each cultivar at the BBCH 65 stage.

RESULTS AND DISCUSSIONS

The following figure shows the average fruit weight for each peach and nectarine cultivar, grown under the Trident and Vertical Axis training systems. This parameter is a key indicator of fruit quality, influenced by genetic factors, crop load, and canopy light distribution. Previous studies have shown that optimized canopy structures improve light interception and fruit uniformity (Anthony and Minas, 2021; Mazzoni et al., 2022). Consistent with these findings, Trident-canopy generally produced slightly heavier fruits, whereas the Vertical Axis

system favored a more uniform fruit size across the canopy.

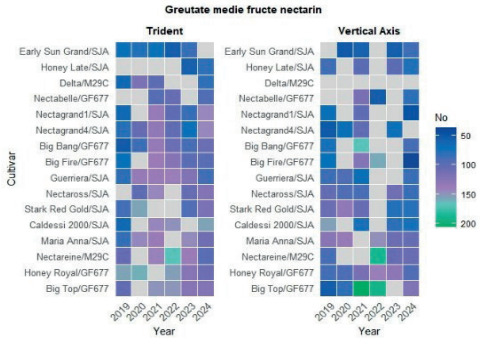


Figure 1. The average fruit weight for nectarine cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

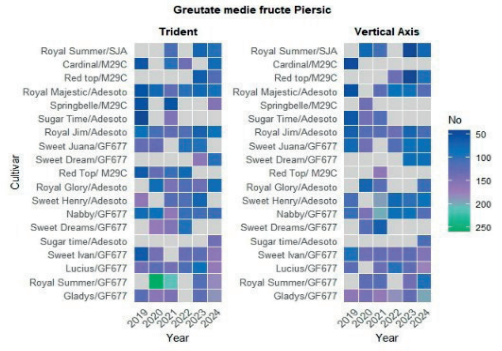


Figure 2. The average fruit weight for peach cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

Nectarine cultivars

The highest average fruit weight on the Trident system was recorded for Nectareine/M29C (164 g-2022) and Honey Royal/GF677' (160.45 g - 2020) cultivars, exceeding over 150 g per fruit. The lowest value was observed for Big Bang/GF677 (85.40 g - 2020) cultivar, below 100 g. On the Vertical Axis, the cultivars Big Top/GF677 (205.05 g - 2021; 191.60 g - 2022), Nectareine/M29C (187.50 g - 2022) showed the largest fruits around 200 g, while Big Fire/GF677(36.5 g - 2024) and Nectagrand 1/SJA (47.05 g - 2024) cultivars, registered the smallest ones, below 50 g.

Peach Cultivars

For the Trident training system, the highest average fruit weight was recorded for the Royal Summer/GF677 (258.67 g - 2020) cultivar, reaching nearly 250 g per fruit, while the lowest value was observed for Royal Jim/Adesoto(87 g - 2024) and Springbelle/M29C (51.88 g - 2019) cultivars, both below 50 g per fruit. Under the Vertical Axis system, the highest fruit weight was obtained for Nabyy/GF677 (164 g - 2021) cultivar, close to 200 g per fuit, whereas the lowest was recorded for Royal Summer/SJA (42.05 g - 2023) cultivar, below 50 g.

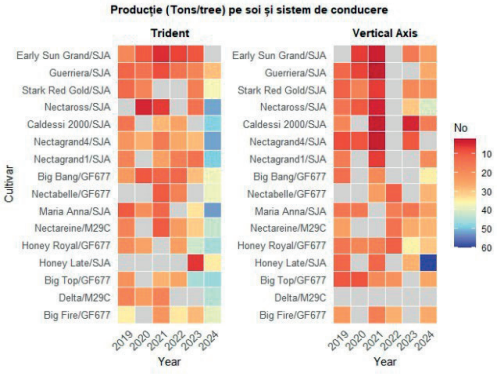


Figure 3. Production (kg/tree) for nectarin cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

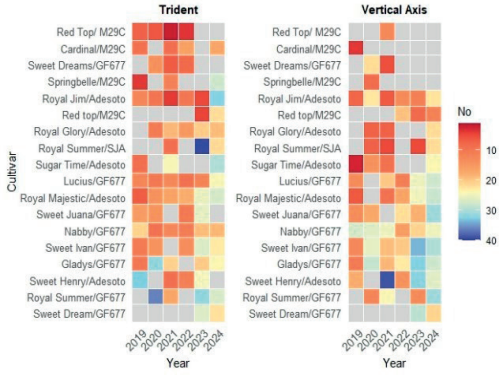


Figure 4. Production (kg/tree) for peach cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

Nectarine cultivars

For the Trident training system, the highest yield per tree was recorded for the Nectaross/SJA, Nectagrand 4/SJA (51.6 kg - 2024) and Maria Anna/SJA (52.3 kg - 2024) cultivars, reaching nearly 50kg/tree, while the lowest value was observed for Honey Late/SJA (5.8 kg - 2023), Nectaross/SJA (4.6 kg - 2020) cultivars, both below 10 kg/tree. Under the Vertical Axis system, the highest highest yield per tree was obtained for Nectaross/SJA (40.4 kg - 2024)

cultivar over 40kg/tree, whereas the lowest was recorded for Caldessi 2000/SJA (2.1 kg - 2021), Guerriera/SJA (2.1 kg - 2021) cultivars, both below 5kg/tree.

Peach Cultivars

For the Trident training system, the highest yield per tree was recorded for the Royal Summer/SJA (39.42 kg - 2023), Royal Summer/GF677 (36.08 kg - 2020), and Royal Jim/Adesoto (32.15 kg - 2024) cultivars, reaching between 30-40 kg/tree, while the lowest value was observed for Red Top/M29C (1.54 kg - 2021), and Springbelle/M29C (3.63 kg - 2019) cultivars, both below 5kg/tree. Under the Vertical Axis system, the highest highest yield per tree was obtained for Sweet Henry/Adesoto (38.95 kg - 2021) cultivars, close 40 kg/tree, whereas the lowest was recorded for Sugar Time/Adesoto (2.36 kg - 2019) and Cardinal/M29C (3.64 kg - 2019) cultivars, both below 10 kg/tree.

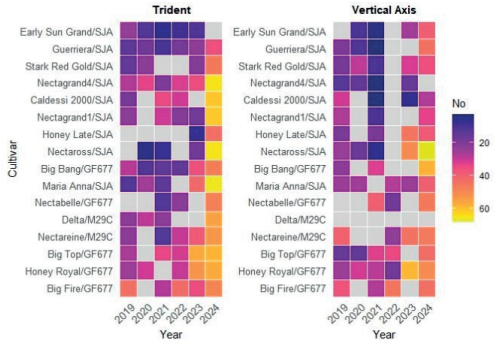


Figure 5. Production (tons/ha) for nectarin cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

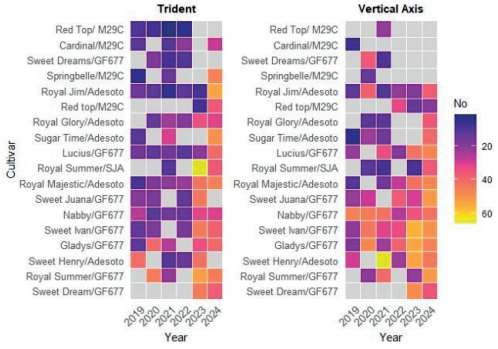


Figure 6. Production (tons/ha) for peach cultivars, grown under the Trident and Vertical Axis training systems (2019-2024)

Nectarine cultivars

For the Trident training system, the highest yield per hectare was recorded for the Nectagrand 1/SJA(61 tons/ha - 2024), Nectaross/SJA (64.47 tons/ha - 2024), and Maria Anna/SJA (65.38 tons/ha - 2024) cultivars, reaching over 60 t/ha, while the lowest value was observed for Nectaross/SJA (5.37 tons/ha - 2020; 7.68 tons/ha - 2021), and Honey Late/SJA (7.24 tons/ha - 2023) cultivars, both below 10 tons/ha. Under the Vertical Axis system, the highest highest yield per hectare was obtained for Nectaross/SJA(67.30-2024), Big Bang/GF677 (58 tons/ha - 2024), Honey Royal/GF677 (58.76 tons/ha - 2023) cultivars, between over 50 tons/ha, whereas the lowest was recorded for Guerriera/SJA (3.51 tons/ha - 2021) and Big Top/GF677 (15.88 tons/ha - 2019) cultivars, both below 15 t/ha.

Peach Cultivars

For the Trident training system, the highest yield per hectare was recorded for the Royal Summer/SJA (65.67 tons/ha - 2023), Royal Summer/GF677 (52.48 tons/ha - 2023), and Sugar Time/Adesoto (49.71 tons/ha - 2024) cultivars, reaching over 50 t/ha, while the lowest value was observed for Springbelle/M29C (4.54 tons/ha - 2019) and Red TopM29C (1.93tons/ha-2022) cultivars, both below 15 t/ha. Under the Vertical Axis system, the highest highest yield per hectare was obtained for Sweet Henry/ Adesoto (64.89 tons/ha - 2021), Sweet/ IvanGF677 (55.88 tons/ha - 2023), Sweet JuanaGF677 (51.40 tons/ha - 2024) cultivars, between over 50 t/ha, whereas the lowest was recorded for Sugar Time/Adesoto (3.93 tons/ha - 2019) and Royal Summer/SJA (8.12 tons/ha - 2023) cultivars, both below 10 t/ha.

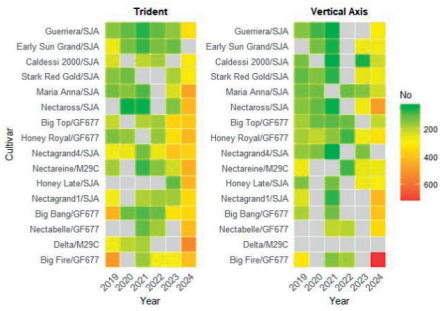


Figure 7. Number of fruits after tinning of nectarin cultivars grown under the Trident and Vertical Axis training systems (2019-2024)

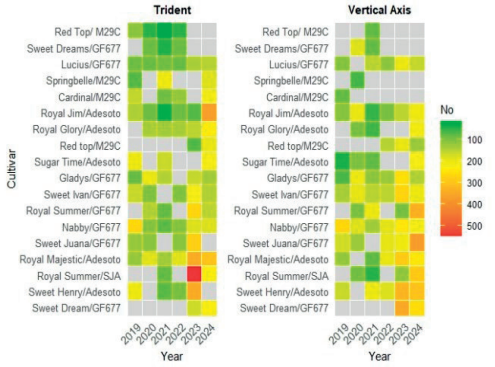


Figure 8. Number of fruits after tinning of peach cultivars grown under the Trident and Vertical Axis training systems (2019-2024)

Nectarine cultivars

For the Trident training system, the highest number of fruits after tinning was recorded for the Delta/M29C (546No. - 2024), Big Bang/GF677 (430No. - 2019), Big Fire/GF677 (500No. - 2019), and Maria Anna/SJA (476No. - 2024) cultivars, reaching over 400 fruits/tree, while the lowest value was observed for Guerriera/SJA(59No. - 2021), and Nectaross/SJA (43No.-2021) cultivars, both below 60 fruits/tree. Under the Vertical Axis system, the highest highest number of fruits after tinning was obtained for Big Fire/GF677 (4714No. - 2024), and Nectagrand4/M29C (447No. - 2024) cultivars, between 400-600 fruits/tree, whereas the lowest was recorded for Nectagrand 4/SJA (21No. - 2021) and Caldessi 200/SJA (29No. - 2021) cultivars, both below 50 fruits/tree.

Peach Cultivars

For the Trident training system, the highest number of fruits after tinning was recorded for the Royal Jim/Adesoto (548No. - 2023) and Royal Majestic/Adesoto (307No. - 2024) cultivars, reaching between 300-500 fruits/tree, while the lowest value was observed for Royal Summer/SJA (45No. - 2021), and Royal Jim/Adesoto (52No. - 2021) cultivars, both below 100 fruits/tree. Under the Vertical Axis system, the highest highest number of fruits after tinning was obtained for Royal Summer/GF677 (336No. - 2024), Sweet Juana/GF677 (374No. - 2024) and Sweet Henry/GF677 (304No. - 2024) cultivars, over 300 fruits/tree, whereas the lowest was recorded for Sugar Time/Adesoto (40No. - 2019) and Royal Majestic/Adesoto (53.02No. - 2019) cultivars, both below 100 fruits/tree.

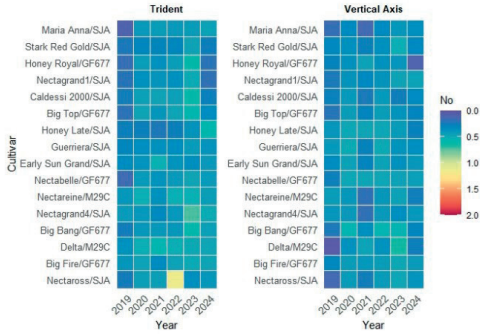


Figure 9. Fruits-flowers rate for nectarin cultivars under the Trident and Vertical Axis training systems (2019-2024)

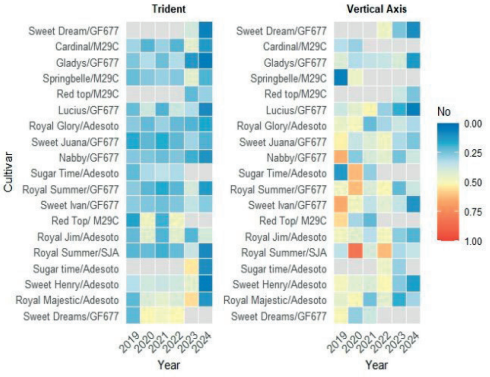


Figure 10. Fruits-flowers rate for peach cultivars under the Trident and Vertical Axis training systems (2019-2024)

Nectarine cultivars

Overral, for the Trident planting system, the fruit-to-flower ratio was optimal for the majority of cultivars, reflecting a high fruit set efficiency under the tested conditions, between 0.7-0.9, while the lowest value was observed for Maria Anna/SJA (8% No - 2019), and Nectabelle/GF677 (15% No - 2019) cultivars,both below 0.5. Under the Vertical Axis system, the fruit-to-flower ratio was optimal for the majority of cultivars, reflecting a high fruit set efficiency under the tested conditions, between 0.7-0.8, while the lowest value was observed for Nectaross/SJA (13% No - 2019), and Delta/M29C (4% No - 2019) cultivars, both below 0.5.

Peach Cultivars

For the Trident planting system, the optimal fruit-to-flower ratio was for Red Top/M29C,(48% No - 2020 - 48% No -2022), Royal Jim/Adesoto(43% No – 2020 -43% No - 2022), Sugar Time/Adesoto(54% No - 2023), and Royal Majestic/Adesoto (54% No - 2023) cultivars, reflecting a fruit set efficiency under the tested conditions, over 0.5, while the lowest value was observed for Sweet Dream/GF677 (6% No -2024), and Sweet Henfry/Adesoto(5% No -2024), Gladys/GF677 (5% No - 2024) both below 0.25. Under the Vertical Axis system, the fruit-to-flower ratio was even better for the majority of cultivars, reflecting a high fruit set efficiency

Royal Summer/SJA (47% No - 2019) and Nabby GF677 (64% No - 2019) rich over 0.9, while the lowest value was observed for Lucius/GF677 (5% No - 2024), and Springbelle/ M29C (6% No - 2019) both below 0.25.

DISSCUSIONS

The results obtained in this study highlight the combined influence of training system, rootstock, and crop management on the productive performance of peach and nectarine cultivars. In general, trees trained under the Vertical Axis system showed a lower average fruit weight compared to those trained under the Trident canopy. This trend was mainly associated with the higher crop load per tree, since fruit thinning was performed less than in normal parameters. Within the Trident system, the cultivar Big Bang/GF677 recorded the lowest fruit weight despite its high genetic potential (over 150 g per fruit), whereas the same cultivar reached higher values under the Vertical(Avilán et al. 1997).

Yield Performance

Regarding yield performance, Royal Summer/GF677 (2020) achieved the highest production per tree and per hectare under the Trident system, confirming its excellent adaptation to vigorous rootstocks and balanced canopy structure. Conversely, Sugar Time/Adesoto and Springbelle (2019) registered the lowest yields, likely due to weaker vegetative growth and reduced fruit set. On the Vertical Axis, Nabyy/GF677 (2021) reached the highest yield, while Royal Summer/GF677 (2023) showed the lowest. Despite the low vigor of the Saint Julien A (SJA) rootstock, which can limit fruit size in some cultivars, results indicate that it can still sustain high yields, demonstrating good adaptability to intensive orchard systems when properly managed(Anthony and Minas, 2021).

Fruit Thinning and Crop Load

The differences between years also underline the importance of fruit thinning practices. In 2021, the number of fruits after thinning reached the lowest levels across all cultivars, a direct consequence of the insufficient thinning performed in 2020, which resulted in tree exhaustion and reduced flower bud formation. This observation reinforces the essential role of adequate crop load management in maintaining fruit size and yield stability over time(Widmer and Krebs, 2001).

Flower-Fruit Ratio

The fruit-to-flower ratio further illustrated the physiological efficiency of the trees. The lowest values were recorded in 2019, when the trees were in their third year after planting, still in the establishment phase with limited fruiting wood(Lauri and Corelli Grappadelli 2014). In contrast, during the following seasons, most cultivars exhibited higher fruit-to-flower ratios, particularly under the Trident system, indicating improved reproductive balance and fruit set efficiency as the orchard matured.

CONCLUSIONS

Overall, these findings demonstrate that the interaction between canopy structure, rootstock vigor, and fruit thinning plays a decisive role in determining both yield potential and fruit quality in modern peach and nectarine orchards. Proper coordination of these factors ensures stable production, optimized fruit size, and long-term orchard sustainability.

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INCIDENCE OF SOME PESTS AND DISEASES ON SEVERAL PEACH AND NECTARINE CULTIVARS TRAINED IN TWO PLANAR CANOPIES

Alexandru BUCUR[1], Ana Cornelia BUTCARU[2], Cosmin Alexandru MIHAI[1], Florin STĂNICĂ[1, 2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

Peach is a popular and important fruit tree widely produced in the world, and producing high-quality peach fruit requires the management of pests and diseases. This study was conducted over seven years (2018-2024) and aimed to examine the incidence of several fungal diseases, such as brown rot (Monilinia fructicola (G. Winter) Honey), powdery mildew (Podosphaera leucotricha (Ellis & Everh.) E.S. Salmon),), and leaf curl disease (Taphrina deformans (Berk.) Tul. and the peach twig borer (Anarsia lineatella Zellfungal) on 16 nectarine and 14 peach cultivars. Trees have been trained on two different canopies: Trident and Vertical Axis. The numbers of mummified fruit and pruned branches on the orchard floor were the main sources of primary inoculum, with Monilinia fructicola for the peach cultivars, while for the Anarsia lineatella, the number of shoots. Visual observations were made for powdery mildew and leaf curl disease.

Key words*: Monilinia fructicola, Anarsia lineatella, Podosphaera leucotricha, canopies, peach.*

INTRODUCTION

Peach cultivation is a key part of global fruit production, but growing high-quality peaches and nectarines comes with its challenges (Manganaris et al., 2022). Orchards need constant care to maintain strong, productive trees, but fungal diseases and insect pests often threaten yields and fruit quality (Anthony & Minas, 2021). Without proper management, these issues can weaken trees, reduce fruit development, and lower market value (Adaskaveg et al., 2023). One of the most damaging fungal diseases is brown rot, caused by (*Monilinia fructicola* (G. Winter) Honey), (Bevacqua et al., 2023). This pathogen spreads quickly and can lead to significant fruit losses both before and after harvest (Navrozidis et al., 2008). Another common issue is powdery mildew (*Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon), which reduces the tree's ability to photosynthesize, weakening shoots and slowing growth (Abdahlla et al., 2024). Peach leaf curl, caused by (*Taphrina deformans* (Berk.) Tul.) is especially problematic in early spring, distorting leaves and affecting overall tree health (Toca et al., 2005; Parveaud et al., 2012). These diseases tend to persist in orchards, often overwintering in mummified fruit or infected wood, making early prevention crucial. Beyond fungal diseases, insect pests like the peach twig borer (*Anarsia lineatella* Zeller) can cause severe damage (Kutinkova et al., 2016; Damos et al., 2022). The larvae tunnel into young shoots and developing fruit, harming the tree and creating entry points for infections (Asfers et al., 2016). How severe these infestations become depends on factors like climate, tree variety, and how well the orchard is maintained. Keeping peach trees healthy requires a balanced approach combining cultural practices, biological solutions, and, when necessary, chemical treatments (Luo et al., 2022). Understanding how these diseases and pests spread helps growers apply the right strategies at the right time. Research into resistant cultivars, improved training systems, and sustainable pest control methods constantly evolves, offering new ways to protect orchards and maintain high fruit quality while reducing reliance on chemical treatments (Maatallah et al., 2024; Anthony & Minas, 2021). In Romania, peach cultivation faces significant challenges, with the most dangerous disease being peach

leaf curl (*Taphrina deformans*), which requires mandatory chemical treatments, and the peach twig borer (*Anarsia lineatella*), as well as the bacterial spot (*Xanthomonas arboricola* pv. *pruni*) (Ivascu & Buciumanu, 2006). To effectively manage these issues, current trends focus on implementing integrated control strategies tailored to local conditions and climate change impacts (Zală et al., 2022). This study aimed to evaluate the incidence of some pests and diseases on several peach and nectarine cultivars over six years (2018-2024). Specifically, it focused on assessing the impact of brown rot (*Monilinia fructicola*), powdery mildew (*Podosphaera leucotricha*), leaf curl disease (*Taphrina deformans*), and the peach twig borer *(Anarsia lineatella)*. Additionally, the study investigated the influence of two different planar canopies, Trident (T) and Vertical axis (Va), on disease severity and pest infestation levels. The research also aimed to identify the primary sources of inoculum of *Monilinia* spp. and the role of orchard sanitation practices in disease and pest management (Jinga et al., 2013; Damianov et al., 2024).

MATERIALS AND METHODS

The research was carried out in the experimental orchard of the Faculty of Horticulture in Bucharest, located at coordinates 44°28′18.14″N and 26°4′13.61″E. The observations were made on two species of stone trees: peach and nectarine. They noted 14 peach and 16 nectarine cultivars (Bucur et al., 2024). Peach trees were approximately 7 years old, planted in 2017, Trident (4.0 x 2.0 m - 1,250 trees/ha - 3,750 axes/ha) and Vertical Axis (4.0 x 1.5 m - 1,666 trees/ha -1,666 axes/ha). Visual inspection is the quickest method for identifying diseases and pests by observing symptoms of infected fruit tree species (Xing et al., 2023). For *A. lineatella*, the number of infested shoots per tree was recorded annually from December 2019 to 2024. Observations were conducted for the pest's first, second, and third generations. In 2020, the CheckMate® SF dispensers were installed in the trees, one at seven trees for mating disruption for management of *A. oriental* fruit moth and peach twig borer in stone fruit crops (Erhaft et al., 2021). For leaf curl disease, each infected leaf on each tree showing visible symptoms of *Taphrina deformans* (Berk.) Tul infection was collected and counted in April 2021-2024. Regarding powdery mildew disease, each affected fruit on each tree shows visible symptoms of *Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon was collected and counted in June 2021-2024. For brown rot (*Monilinia fructicola* (G. Winter) Honey), the number of mummified fruits left on the tree from each tree was counted in December 2018-2024. The orchard was maintained under a conventional treatment scheme. The phytosanitary treatment scheme for the peach and nectarine experimental orchard at USAMV of Bucharest includes strategic applications throughout the year, targeting major fungal and bacterial diseases and key insect pests. Treatments were applied from January to December, following the phenological stages of the crop based on the BBCH scale. A combination of fungicides, bactericides, and insecticides, including Dithane M45, Captan, Syllit 400 SC, Score 250 EC, Vertimec 1.8 EC, Movento 100 SC, and other protective substances, was used. The main diseases controlled were brown rot (*Monilinia fructicola* (G. Winter) Honey), bacterial spot (*Xanthomonas arboricola* pv. *pruni*), powdery mildew (*Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon), leaf curl (*Taphrina deformans*), and others. Additionally, treatments aimed to mitigate pest attacks. Treatments were applied in pre- and post-harvest periods, during dormancy and active growth, ensuring crop protection.

For data statistical analysis, the R program (with RStudio 2024.04.2+764) and MS Office (Microsoft Excel, 2016) were used, with a significance level of $p = 0.05$, for the descriptive statistics of the data. ANOVA and Tukey post-hoc tests were applied.

RESULTS AND DISCUSSIONS

Comparing peach and nectarine of *Anarsia lineatella* attack, shows that the nectarine trees exhibited a more severe initial infestation compared to peaches, with certain cultivars ('Nectabelle/GF677') showing over 200 infested shoots per tree in 2019. Following the installation of CheckMate® SF Dispenser, a major decrease in infestation was recorded across both peach and nectarine cultivars. By

2021, the number of infested shoots dropped to near-zero levels in most cultivars, and this trend continued through 2024. Most susceptible cultivars (with the highest infestation levels) in peach were 'Sugar Time/Adesoto' (11.56%) (Figure 2), and for nectarine, 'Nectabelle/GF677' (48.58 %) (Figure 1). Also, these cultivars exhibited the highest attack levels before the application of mating disruption, indicating a high susceptibility to *A. lineatella*. Most tolerant cultivars (lowest infestation levels) in peach, were 'Cardinal/M29C' (T) (0.67%) (Figure 2), and for nectarine, 'Nectareine/M29C' (Va) (1.11%), but trained on Trident 'Nectareine/M29C' was registered with (13.83%) infection level (Figure 1). These cultivars maintained the lowest infestation rates throughout the study period, suggesting a degree of resistance. The 'Royal Summer/GF677' (6.17%), cv./rootstocks combination exhibited higher infection levels than the 'Royal Summer/SJA' (1.33%) on attacked shoots (2019-2024) (Figure 2). Regarding Trident canopy, the most susceptible cultivar in peach was 'Sugar Time/Adesoto' (11.56%), and the most tolerant was 'Royal Summer/SJA' (1.33%) (Figure 3). For the Vertical axis, the most susceptible cultivar was 'Gladys' (4.22%), and the most tolerant was 'Cardinal/M29C' (0.67%) (Figure 3). For the nectarine cultivars, trained on Trident canopy, the most susceptible cultivar was 'Nectabelle/GF677' (48.58%), and the most tolerant was 'Nectagrand 4/SJA' (9.00%) (Figure 4). For the Vertical axis, the most susceptible cultivar was 'Big Bang/GF677' (6.39%), and the most tolerant was 'Nectareine/M29C' (1.11%) (Figure 4). Relating to observations for the first, second, and third generations of the pest (Figure 5), the results for the nectarine cultivars trained on Trident canopy showed higher levels of infestation, particularly in early years (2019-2020) and especially in generation two. The Vertical axis canopy exhibited a lower infestation, with the least minimal attack. Overall, some cultivars such as 'Nectabelle/GF677', 'Guerriera/SJA', 'Early Sun Grand/SJA', 'Big Bang/GF677', 'Big Top/GF677', and 'Big Fire/GF677' appear to be more vulnerable. Other cultivars such as 'Maria Anna/SJA', 'Nectagrand 4/SJA', and 'Caldessi2000/SJA' showed consistently lower infestations. For peach cultivars, attack based on generations was the same as that of nectarine cultivars, with much lower intensity overall. In the first generation, the attack levels were generally low across all years, with very few occurrences of moderate infestation. Under the Trident canopy, the second generation was the most severe attack (Figure 6). In the third generation, the attack level was minimal across all years, with little to no impact. Some cultivars, such as 'Sugartime/Adesoto', 'Sweet Henry/Adesoto', 'Lucius/GF677', and 'Gladys/GF677', expressed higher attack, and the attack levels on the cultivars 'Royal Summers/SJA', 'Springbelle/M29C', and 'Red Top was low.

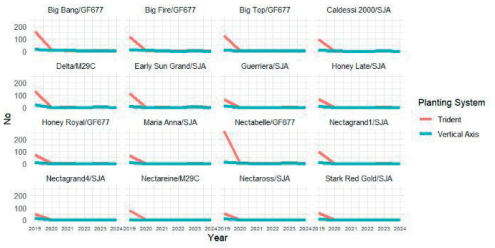


Figure 1. *Anarsia lineatella* attack of shoots in nectarine cultivars (2019-2024)

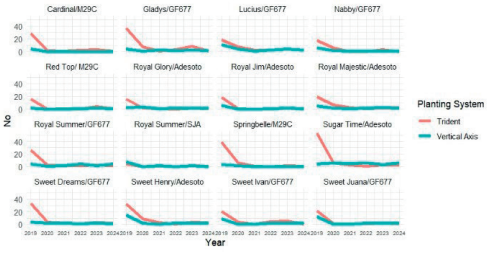


Figure 2. *Anarsia lineatella* attack on shoots in peach cultivars (2019-2024)

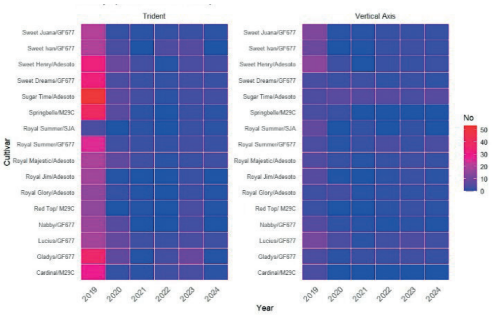


Figure 3. The evolution of *Anarsia lineatella* attack in peach cultivars trained under two different planar canopies (T-Va)

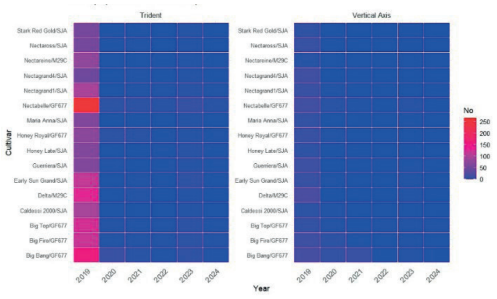


Figure 4. The evolution of *Anarsia lineatella* attack in nectarine cultivars trained under two different planar canopies (T-Va)

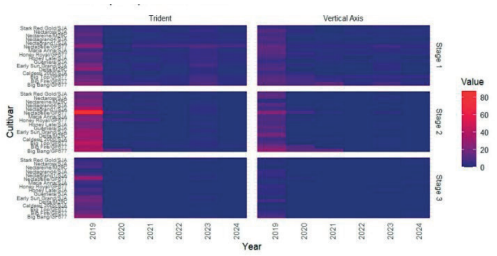


Figure 5. Observations in nectarine cultivars for the pest's first, second, and third generations regarding two canopies (2019-2024)

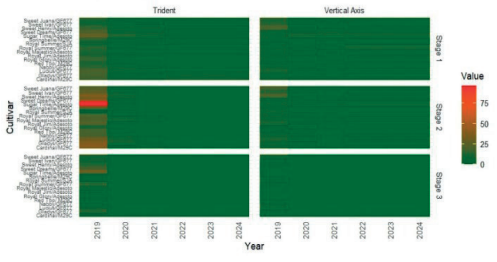


Figure 6. Observations in peach cultivars for the pest's first, second, and third generations regarding the two canopies (2019-2024)

Taphrina deformans had been present in peach and nectarine since the end of March and early April. Leaf curl disease appeared on young leaves, shoots, and sometimes on fruits. A clear distinction was observed between the two canopies, with the Trident canopy exhibiting higher infection levels in most cultivars than the Vertical axis canopy. This trend was particularly evident in the first two years (2021-2022), where the number of infected leaves was significantly greater under the Trident canopy. Among nectarine cultivars, 'Big Top/GF677' (T) (1005 infected leaves) exhibited the highest infection levels (Figure 7).

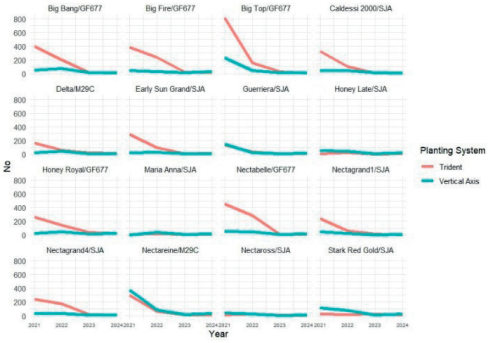


Figure 7. *Taphrina deformans* infestation in nectarine cultivars (2021-2024)

'Lucius/GF677' (Va) (431 infected leaves) recorded the most severe infections for the peach cultivars. Moreover, the lowest infection rates were recorded on 'Maria Anna/SJA' cv. (T) (37 infected leaves)-(nectarine) and 'Red Top/M29C' (Va) (35 infected leaves)-(peach) (Figure 8).

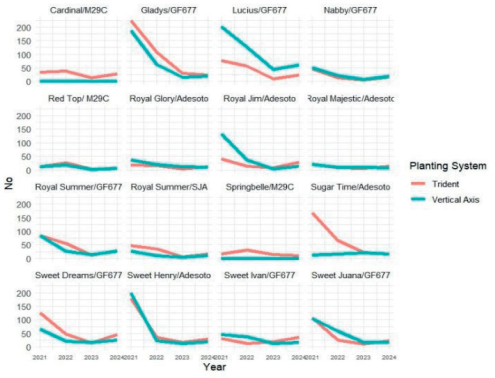


Figure 8. *Taphrina deformans* cultivars infestation in peach cultivars (2021-2024)

For the Trident canopy, the most susceptible nectarine cultivar was 'Big Top/GF677' (1005 infected leaves), and the most tolerant was 'Maria Anna/SJA' (37 infected leaves) (Figure 9). For the Vertical axis, the most susceptible nectarine cultivar was 'Nectareine/Adesoto' (516 infected leaves), and the most tolerant was 'Early Sun Grand/SJA' (66 infected leaves) (Figure 9). Regarding the Trident canopy, the most susceptible peach cultivar was 'Gladys/GF677' (384 infected leaves), and the most tolerant was 'Red Top/M29C' (47 infected leaves) (Figure 10).

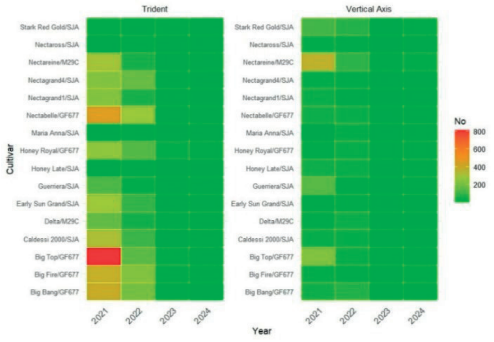


Figure 9. The evolution of *Taphrina deformans* infestation in nectarine cv. trained under two different planar canopies (2021-2024)

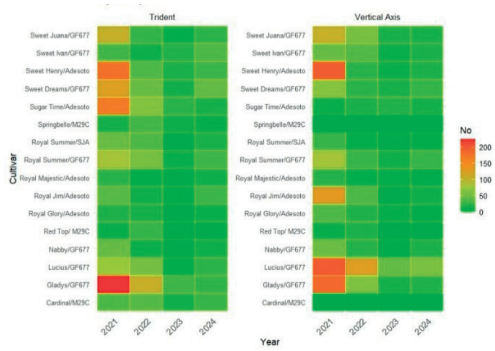


Figure 10. The evolution of *Taphrina deformans* infestation in peach cv. trained under two different planar canopies (2021-2024)

For the Vertical axis, the most susceptible cultivar was 'Lucius/GF677' (431 infected leaves), and the most tolerant was 'Red Top/M29C' (65 infected leaves) (Figure 10). The 'Royal Summer/GF677' cv./combination again showed higher infection than the 'Royal Summer/SJA' combination regarding *Taphrina deformans* (Figure 10).

Powdery mildew has been present mostly in nectarine cultivars since mid-May (Figure 11). The severity of *(Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon) infection varied depending on the training system. In the Trident canopy (Figure 13), the highest infection level was observed in 'Guerriera/SJA' (35,5%) while the lowest was recorded in 'Nectagrand 1/SJA' (6.50%). In the Vertical Axis canopy (Figure 13), 'Nectareine/Adesoto' (35.00 %) showed the highest susceptibility, whereas 'Nectagrand1/SJA' (6.50 %) showed the lowest infection levels. The annual distribution of the infection rate under the two planar canopies was higher in 2023, with no significant differences regarding the canopies (Figure 12).



Figure 11. *Podosphaera leucotricha* infestation in nectarine cultivars (2020-2024)

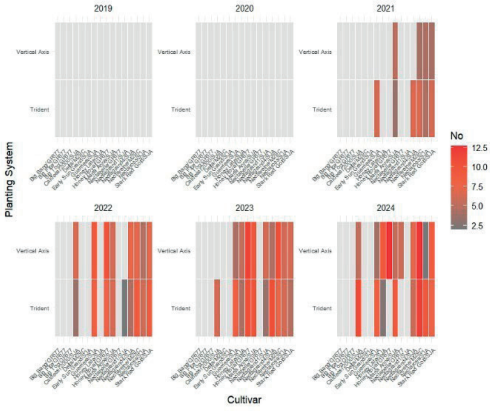


Figure 12. Annual distribution of powdery mildew (*Podosphaera pannosa*) infestation in nectarine cultivars under different canopies (2019-2024)

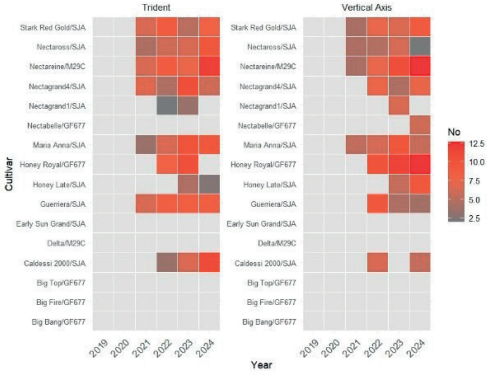


Figure 13. Incidence of powdery mildew (*Podosphaera pannosa*) in nectarine cultivars under Trident and Vertical Axis training systems (2019-2024)

Monilinia fructicola (G. Winter) Honey felt its presence in peach and nectarine at the end of the ripening stage of the fruits, mostly in late-ripening cultivars. The incidence of *Monilinia* spp. infection varied among peach and nectarine cultivars, with significant differences observed between the Trident and Vertical Axis canopies. The highest infection levels in peaches were recorded in 'Gladys/GF677' (T) (10.2%), showing a progressive increase in disease incidence, particularly from 2022 to 2024. Similarly, 'Lucius/GF677' (Va) (9.5%) exhibited consistently high infection rates, and the lowest infection levels were in 'Royal Summer/SJA' (T) (3.5%) (Figure 14).

In nectarines, the highest infection levels were recorded in 'Nectareine/Adesoto' (Va) (7.4%), and the lowest infection levels were 'Caldessi2000/SJA' (T) (3.3%) (Figure 15).



Figure 14. *Monilinia fructicola* infestation in peach cultivars (2019-2024)

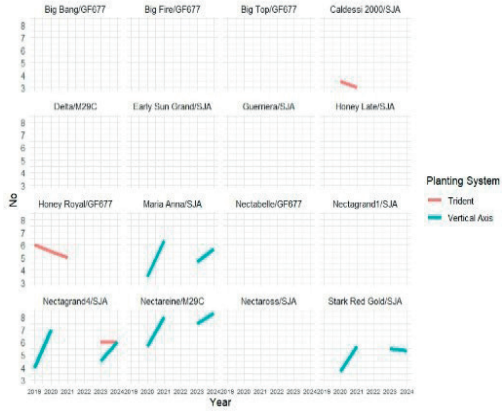


Figure 15. *Monilinia fructicola* infestation in nectarine cultivars (2019-2024)

For the Trident canopy, the lowest infection levels in peaches were observed in 'Red Top/M29C' (2.5%), which maintained relatively low disease incidence, and the highest infection levels were observed in 'Gladys/GF677' (10.2%). For the Vertical Axis canopy, the lowest infection levels were observed in 'Royal Jim/Adesoto' (4.5%), and the highest infection levels were observed in 'Lucius/GF677' (9.4%) (Figure 17). For nectarines, under the Trident canopy the most affected cultivars were 'Guerriera/SJA' (6.5%), the lowest infection levels was observed in 'Caldessi 2000/SJA' (3.3%), and for the Vertical Axis canopy, which exhibited a significant increase in disease incidence from 2022 the cultivars with the lowest infection levels were 'Guerriera/SJA', 'Necatross/SJA' (3.5%) and the highest infection levels was in 'Nectareine/Adesoto' (7.4%) (Figure 16).



Figure16. Incidence of *Monilinia* Infection in nectarine cultivars under Trident and Vertical Axis canopies (2019-2024)



Figure 17. Incidence of *Monilinia* infection in peach cultivars under Trident and Vertical Axis canopies (2019-2024)

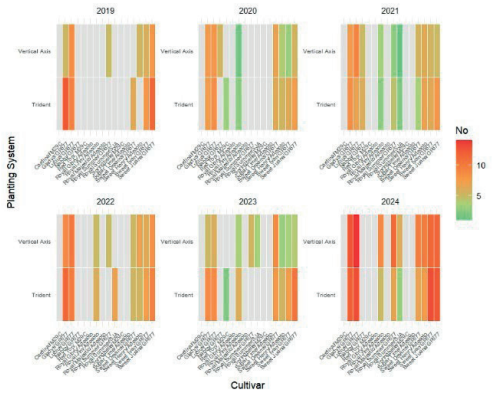


Figure 18. Yearly evolution of *Monilinia* infection in peach and nectarine cultivars under different training systems (2019-2024)

CONCLUSIONS

The study revealed the presence and variability of several diseases: *Taphrina deformans, Podosphaera leucotrica, Monilinia fruticola*, and pest: *Anarsia lineatella* generations attacks in peach and nectarine cultivars under natural infection conditions over the study period (2019-2024) (Rossi et al., 2007; Thomidis & Paresidou, 2023; Rossi et al., 2006). The intensity of disease and pest incidence was influenced by cultivar susceptibility, training system, and climatic factors, despite applying phytosanitary treatments (Zală et al., 2022; Costa et al., 2006). The increased shoot density and greater vegetative growth in the Trident canopy may have provided a more favorable microclimate for pest development and facilitated higher infestation levels for *Anarsia lineatella* attack. The presence of *A. lineatella* was detected across multiple cultivars, with 'Royal Summer/GF677' showing the highest infestation levels compared to the 'Royal Summer/SJA' overall. Moreover, the continued presence of *A. lineatella* in the second generation highlighted the importance of integrated pest management (IPM) strategies, including biological control methods, pheromone traps, and improved monitoring techniques (Abdahlla et al., 2024). The Trident system generally exhibited higher infection rates, particularly for *Taphrina deformans* and *Monilinia fruticola*, with cultivars such as 'Royal Summer/GF677' (peach), 'Big Top/GF677' (nectarine), and 'Gladys/GF677' (peach) being among the most affected. In contrast, the Vertical Axis canopy showed lower disease incidence, with cultivars like 'Nectaross/SJA' (nectarine) and 'Royal Summer/SJA' (peach) displaying greater tolerance. However, some cultivars 'Nectarine/M29C' and 'Lucius/GF677' recorded increased infection in 2024, suggesting the influence of environmental conditions and disease progression. For powdery mildew (*Podosphaera leucotrica*), the highest levels were recorded in 'Guerriera/SJA' (T) and 'Nectareine/M29C' (Va), while 'Nectagrand 1' (T/Va) exhibited the lowest incidence. Future disease and pest control measures should focus on: Adopting climate-resilient disease management techniques, particularly for *Monilinia* and *Taphrina deformans*; Enhancing IPM approaches for *Anarsia lineatella*, integrating biological control agents and cultural practices to reduce infestation levels sustainably.

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BEHAVIOR OF SOME BLUEBERRY VARIETIES ON DIFFERENT GROWING SYSTEMS IN THE BUCHAREST AREA

Mihaela BUJOR[1], Adrian ASĂNICĂ[1], Valerica TUDOR[1], Dan POPESCU[1], Oana Vasilica HERA[2], Răzvan Ionuț TEODORESCU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing Pitești - Mărăcineni, 402 Mărului Street, Mărăcineni, Argeș County, Romania

Corresponding author email: adrian.asanica@horticultura-bucuresti.ro

Abstract

In Romania, interest in blueberry cultivation has increased significantly in recent years, due to the growing market demand for this nutrient-rich and antioxidant-packed fruit. Thus, cultivating blueberries can not only bring substantial economic benefits, but also contribute to environmental protection and to improving consumer health. This makes blueberries a particularly attractive choice for farmers and investors, as well as a smart and advantageous option from multiple perspectives: economic, ecological, and health-related. The objective of this study was to evaluate the main growth and fruiting characteristics of 17 blueberry cultivars (Vaccinium corymbosum L.) grown in the Bucharest area, in two ridge cultivation systems: trellised and free (no wired). The correlation analysis highlights the fact that the trellised system provides a much more coherent relationship between vegetative parameters (no of stems, shoots, and buds) and fruiting potential, suggesting a superior and more efficient use of the plant's physiological resources. At the same time, we seized that the total yield is not strictly determined by the number of fruits, reflecting differences among cultivars in terms of fruit size and average fruit weight. This aspect and results could be explained by the fact that the analysed plants are still young and have not yet reached their maximum biological fruiting potential.

Key words*: Vaccinium corymbosum L.,* trellising, growing system, productivity.

INTRODUCTION

Blueberry belongs to the Ericaceae family, genus *Vaccinium*, which includes over 200 wild and cultivated species. The bog blueberry (*Vaccinium uliginosum* L.), the bilberry (*Vaccinium myrtillus* L.), and the lingonberry (*Vaccinium vitis-idaea* L.) are among the spontaneous species found in Romania, especially in hilly pre-mountain and mountain areas. They grow as low shrubs (between 20-50 cm tall, and very rarely up to 80 cm), with creeping stems and small fruits. Cultivated blueberry varieties (highbush types) - tall shrubs that can reach 1-3 meters in height - produce fruits that are two to four times larger than those of wild blueberries, originating mostly from three American species: *Vaccinium corymbosum* (primarily), *Vaccinium lamarckii*, and *Vaccinium angustifolia*.

Blueberries are among the most popular fruits in North America, being rich in anthocyanins, which have beneficial health effects against several chronic diseases, including cardio-vascular disorders, neurodegenerative diseases, diabetes, and cancer (Routray & Orsat, 2011).

In Romania, the interest in blueberry cultivation has increased significantly in recent years due to the rising market demand for this nutrient-and antioxidant-rich fruit. An important aspect is the presence of European funding programs that support farmers in establishing and developing blueberry plantations. Access to EU funds has stimulated investment in this crop, and Romania currently reports over 2,200 hectares (Asănică et al., 2017; IBO report, 2025). Blueberry cultivation is of great importance today due to the high antioxidant content of the fruits and their contribution to a healthy diet (Asănică et al., 2023). Thus, blueberry cultivation can not only generate significant economic benefits but also contribute to environmental protection and improved consumer health. This makes blueberries an especially attractive option for farmers and investors, as well as a smart and

advantageous choice from economic, ecological, and health perspectives.
Fruit production may be delayed by climatic characteristics, and some years can significantly influence the progression of blueberry phenophases, influencing the quality of compounds accumulated in the fruits.
The results presented are important for farmers, providing the information needed to cultivate varieties that are preferred by consumers (Ojog et al., 2024), in close correlation with the phenotypic plasticity specific to each variety in response to gradual changes in environmental conditions (Cosmulescu et al., 2022). An innovative study by Asănică et al. (2023) proposed an upright training system for blueberries by transforming the natural bush architecture into one with multiple vertical axes supported by wires and posts. Results for the 'Coville' and 'Blueray' cultivars showed that although total yield per bush was slightly higher than in the traditional system, the fruits obtained in the vertical system had superior quality (larger berries, improved color), more uniform fruit set, easier harvesting, and better light utilization - all beneficial for small farms. Large differences among cultivars in growth, soluble sugar content, and antioxidant enzyme activity were found when testing 15 cultivars for tolerance to high soil pH, identifying groups of varieties with high pH tolerance (Hao et al., 2022). Alternatives to traditional open-field production include protected environments, greenhouses, high tunnels, high-density plantings, plant production systems, and pot production. All these systems have demonstrated the potential to produce high yields of top-quality berries. Recently, pot blueberry production using soilless substrates has attracted increasing interest as a viable alternative to open-field planting (Smrke et al., 2022).

MATERIALS AND METHODS

In this paper, 17 blueberry varieties (*Vaccinium corymbosum* L.) were analysed from the Experimental Field of the Faculty of Horticulture (Figure 1) in Bucharest, within the blueberry variety collection established in 2018, in two ridge cultivation systems: trellised and untrellised, with 5 plants per cultivar.



Figure 1. Experimental field of the Faculty of Horticulture

A planting distance of 3 × 0.8 m, with raised beds covered with agrotextile, and a drip irrigation system (each plant having two drippers) was provided to the experimental plot.



Figure 2. Planting blueberries on raised beds

For planting, a quantity of 60 liters of acidic peat (Kekkila FBM 525) was calculated for each plant, and in the process, the peat was mixed with the existing substrate using a rotary tiller before the ridge was formed (Figure 2). The planting layout included 17 blueberry cultivars arranged in two mirror rows, each row containing 85 plants. The trellising system was realised using concrete poles installed along the length of the row, while the trellising of the blueberry stems was carried out using a system of wires positioned laterally at two levels, 45 cm from the bed and 100 cm the second layer.
In 2023, the plants were trellised using fastening clips attached to the supporting wires.
To evaluate the 17 blueberry varieties behaviour, determinations were made on the following quantitative and qualitative characteristics of the main growth and fruiting traits (Cociu & Oprea, 1989):

• average number of stems per plant;
• average length of annual shoots (cm) measured by assessing all shoots;
• number of fruit buds before bud break;
• number of fruits set before entering in the ripening stage;
• average fruit yield (g/plant).

Natural conditions of the research area.

The Municipality of Bucharest is located in the Vlăsia Plain, the central subdivision of the Romanian Plain. It is bordered by the Titu–Gherghița Plain to the North, the Bărăganul Mostiștei (or Southern Bărăgan) Plain to the east and South-East, the middle course of the Ialomița River to the North-East, and the lower Argeș River valley to the West and South-West. The plain is typically tabular, with wide and smooth interfluves.

The geological foundation consists of Proterozoic crystalline deposits belonging to the Wallachian Platform. Above this foundation lie Paleozoic and Mesozoic sedimentary layers, which are overlain by Quaternary surface materials. These include 2-12 m thick loess layers, within which numerous depressions have formed through settlement and suffosion processes. The Bucharest Plain, a subunit of the Vlăsia Plain, has altitudes ranging from 100-115 m in the northwest to 50-60 m in the southeast, in the Dâmbovița floodplain. The city itself varies between 58 m and 90 m in elevation, while the Băneasa (or Otopeni) Field, located North of the Colentina Valley, reaches 90-95 m. Overall, the terrain has the appearance of a gently undulating plain.

The climate is temperate-continental, characterized by moderate thermal extremes caused by high urban density, heavy traffic, and industrial activities. While the annual mean temperature is 10.7°C in peripheral areas, the central zone records an average of 12°C. In climatologically normal years, summers are warm - sometimes hot - and often marked by drought, while winters are cold and with abundant snowfall, especially in the northern districts. Springs are short, with large temperature fluctuations from month to month and significant differences between day and night. Autumn is characterized by moderate temperatures and a gradual transition toward winter. The frost-free interval of 178-205 days and the growing season of about 245 days are generally favourable for cereal crops as well as various fruit, vegetable, and ornamental species.

Annual precipitation ranges between 550 and 600 mm, with maxima recorded in June-July. Showers and torrential rains are frequent. Dominant air circulation comes from the east and northeast during winter and from the west in the rest of the year. Maximum wind speeds range between 4-5 m/s.

Soil pedological and agrochemical characterization.

The predominant soil in the study area is a reddish preluvosol, characterized by a low humus content of 2.5%, a loam-clay texture, a pH of 6.2, and low to medium nutrient supply (N, P, K). Blueberry requires medium-textured, well-drained soils without gleying to a depth of 40–50 cm and an acidic reaction. The optimal soil pH range is between 4.3 and 5.5. Lowering soil pH before planting and maintaining healthy plants is essential. Soil acidification is typically achieved by adding elemental sulfur or other sulfur-based amendments (approximately 250 kg sulfur/ha to decrease soil pH by one unit). For acidifying soils intended for blueberry cultivation, the soil must be mixed with acidic peat at a 1:1 ratio to a depth of 15-30 cm.

Data interpretation.

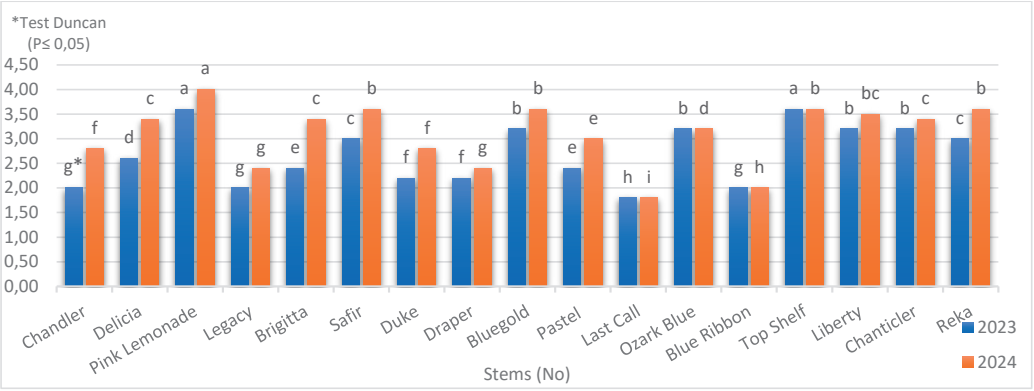
Statistical analysis was performed using IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were statistically evaluated by analysis of variance (ANOVA). Differences between cultivars were highlighted through Duncan's multiple test range ($p< 0.005$). Graphical representations were performed with Microsoft Office Excel 2007.

RESULTS AND DISCUSSIONS

The comparative analysis conducted for the two growing systems, untrellising vs. trellising, was carried out over two years (2023 and 2024) for the following growth and fruiting characteristics, together with a simple correlation analysis between growth and fruiting traits.

Average number of stems per plant

For the untrellising system, the values ranged from 1.8 stems ('Last Call') to 3.6 stems ('Top Shelf', 'Pink Lemonade') in 2023, and from 1.8 stems ('Last Call') to 4.0 stems ('Pink Lemonade') in 2024 (Figure 3).

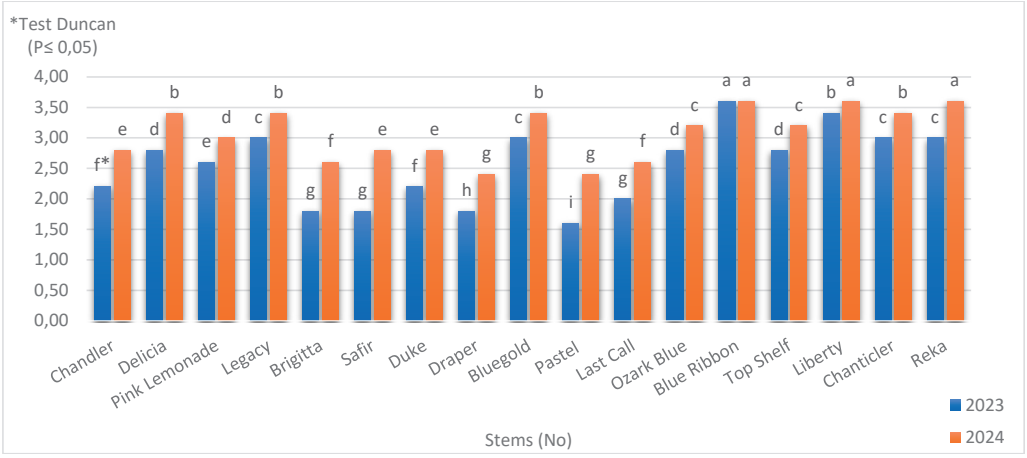


*Different letters between cultivars denote significant differences (Duncan test. p < 0.05)

Figure 3. Influence of the untrellised system on the average number of stems/plant

In the trellising system, vegetative growth was slightly lower in the first year, ranging between 1.6-3.6 stems, but became more uniform in 2024 (range: 2.4-3.6 stems) (Figure 4).

The ANOVA statistical analysis ($\alpha = 0.05$) shows that the differences among cultivars are not statistically significant ($p > 0.05$). However, the biological tendency toward increased stem production in cultivars with high vegetative potential ('Reka', 'Top Shelf', 'Pink Lemonade') is evident.

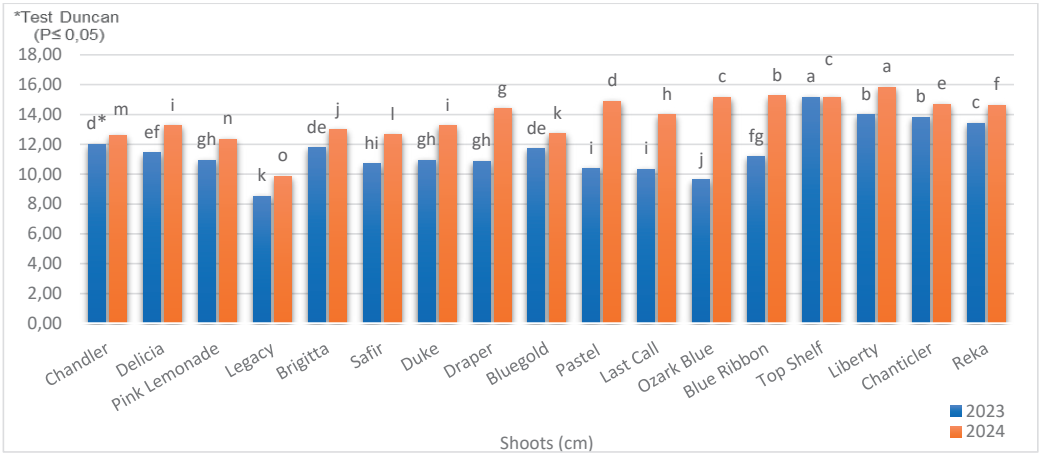


*Different letters between cultivars denote significant differences (Duncan test. p < 0.05)

Figure 4. Influence of the trellising system on the average number of stems/plant

Average length of annual shoots (cm)

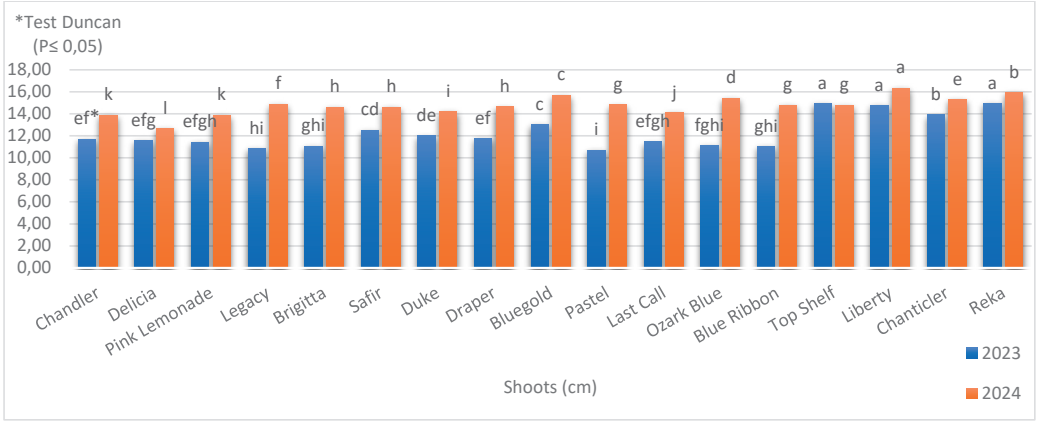
For the bush free type (non-trellised) training system, the Duncan test grouped the studied cultivars into 11 classes for 2023 and 15 classes for 2024, while for the trellised system the grouping ranged between 9 and 12 classes. In the non-trellised variant, the average shoot length increased from 8.5 cm ('Legacy') to 15.17 cm ('Top Shelf') in 2023, and in 2024 from 9.87 cm ('Legacy') to 15.81 cm ('Liberty') (Figure 5).



*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 5. Influence of the untrellised system on the average length of shoots (cm/plant)

In the trellising variant, the values were higher, reaching in 2024 a maximum of 16.32 cm ('Liberty') (Figure 6). The more vigorous cultivars respond better to trellising, which optimizes light exposure and ensures the balanced development of the vegetative growths.

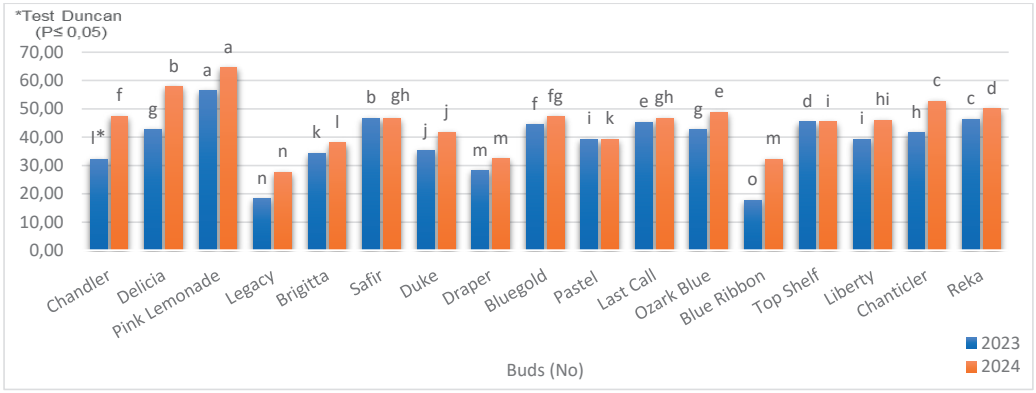


*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 6. Influence of the trellising system on the average length of shoots (cm/plant)

Number of fruit buds

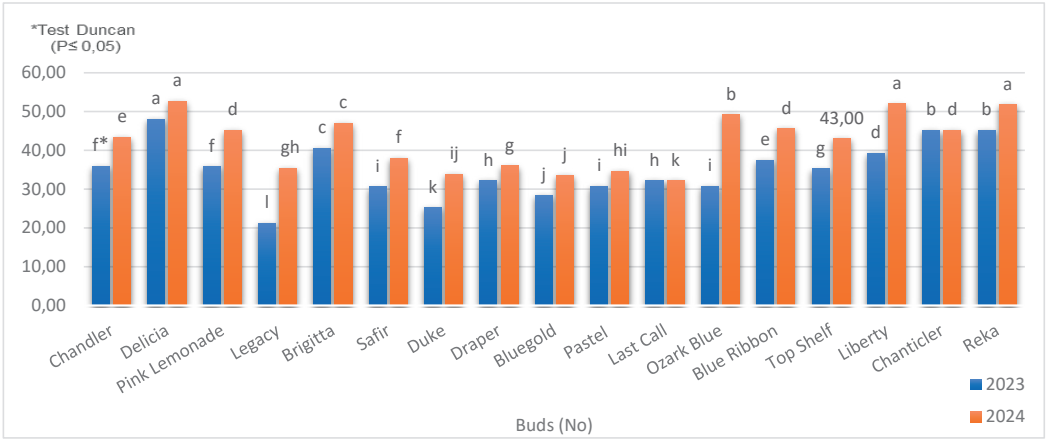
From the analysis of Figure 5, it can be observed that the average number of fruit buds per plant increased significantly from 17.8 buds ('Blue Ribbon', untrellised, 2023) to 64.6 buds ('Pink Lemonade', untrellised, 2024) (Figure 7).



*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 7. Influence of the untrellised system on the average number of fruit buds/plant

In the trellising system, more balanced values were obtained (21-52 buds per plant in 2024) (Figure 8), indicating a significant difference between the training systems ($p < 0.05$). This demonstrates that trellising leads to a more uniform distribution of buds, reducing yield alternation. Cultivars with high productive potential ('Pink Lemonade', 'Reka', 'Delicia') benefit more from controlled plant architecture, which favours the formation of floral buds.

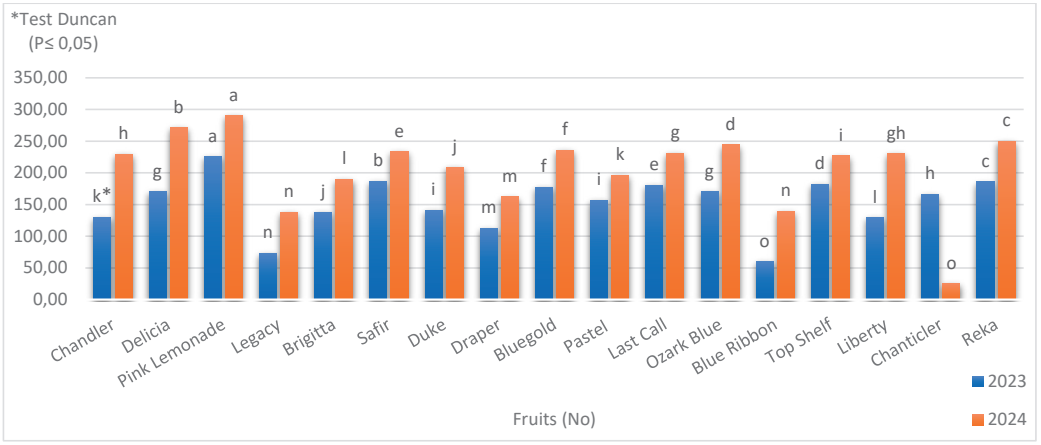


*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 8. Influence of the trellising system on the average number of fruit buds/plant

Number of fruits set

Regarding the characteristic average number of fruits per plant, the data show a clear increase in productive potential in both systems. In the untrellised variant, the values increased from 60 fruits ('Blue Ribbon') in 2023 to 290 fruits ('Pink Lemonade') in 2024 (Figure 9).

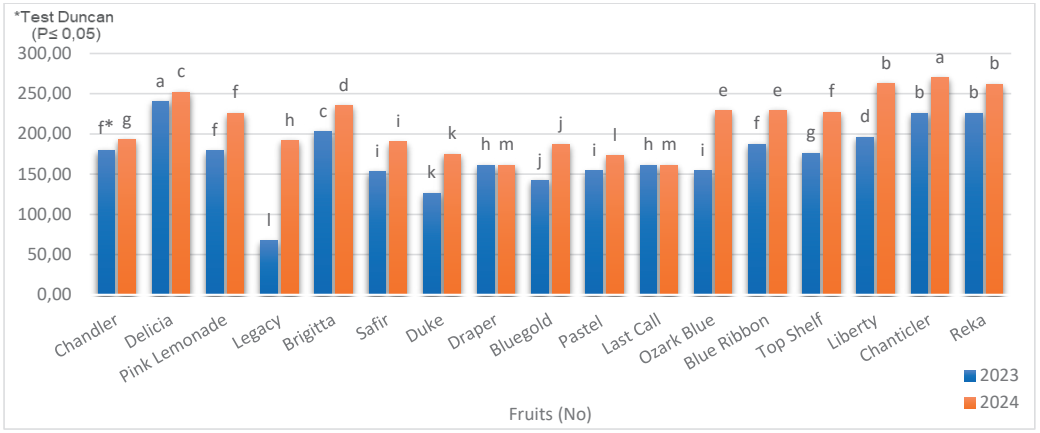


*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 9. Influence of the untrellised system on the average number of fruits/plant

In the trellising variant, more uniform yields were recorded, ranging from 67 fruits ('Legacy') to 269 fruits ('Chanticler') in 2023, and reaching approximately 270 fruits ('Delicia') in 2024 (Figure 10).

Although the variation among cultivars is significant, the Duncan test shows overlaps between significance classes ($p > 0.05$ for some cultivars), suggesting a complex interaction between cultivar and the chosen growing system.



*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 10. Influence of the trellising system on the average number of fruits/plant

Average fruit yield per plant

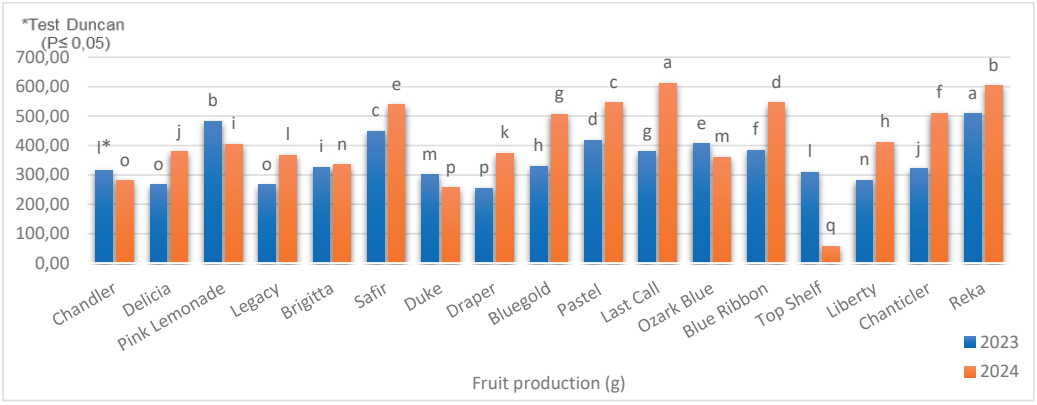
From the perspective of average yield per plant, the ANOVA analysis confirms significant differences between years and training systems ($p < 0.01$). Thus, the cultivars 'Reka' (382 g) in 2023 and 'Liberty' (505 g) in 2024 recorded the highest yields (g) in the untrellised system (Figure 11).



*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 11. Influence of the untrellised system on average production (g)/plant

In the trellising variant, the values were significantly higher, reaching up to 382 g ('Reka') in 2023 and 612 g per plant ('Last Call') in 2024. Trellising had a significant positive effect on yield by optimizing fruit distribution, light exposure, and photosynthetic efficiency (Figure 12).



*Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$)

Figure 12. Influence of the trellising system on average production (g)/plant

Simple correlation analysis between growth and fruiting traits in blueberry (*V. corymbosum* L.) (Table 1)

1. Untrellising system (2023)

The results indicate the presence of positive and significant correlations between the number of stems and the number of shoots ($r = 0.515^{**}$) as well as between the number of shoots and the number of buds ($r = 0.320^{*}$). A very strong correlation was also observed between the number of buds and the number of fruits ($r = 0.986^{**}$, $p < 0.01$), confirming the direct dependence between flowering potential and fruit set level.

The moderate positive correlation between cultivar and the number of stems ($r = 0.331^{*}$) and between cultivar and the number of shoots ($r = 0.525^{**}$) suggests significant genetic variation among cultivars regarding vegetative potential.

Fruit yield did not show a significant correlation with the other morpho-vegetative parameters, indicating a strong influence of external factors (climatic and edaphic conditions) on the final yield in the untrellising system.

2. Untrellising system (2024)

In 2024, the correlation structure changed slightly. The relationship between cultivar and shoot length was strongly positive (r = 0.764**, p < 0.01), confirming the genetic stability of this trait. Additionally, the number of stems was significantly correlated with the number of buds (r = 0.647**, p < 0.01), suggesting that vegetative vigour promotes better formation of fruit buds.

The negative correlations observed between cultivar and number of fruits (r = –0.310*) or between number of shoots and number of fruits (r = –0.064) indicate a possible competition between vegetative growth and fruiting, common in young or vigorous plants.

3. Trellising system (2023)

In the trellising system, relationships between growth traits were stronger. A significant correlation was recorded between cultivar and number of shoots (r = 0.626**, p < 0.01), as well as between the number of shoots and the number of buds (r = 0.378**, p < 0.01).

The very strong relationship between the number of buds and the number of fruits (r = 0.979**) shows that in the trellising system, fruiting closely follows flowering potential, suggesting high efficiency in pollination and fruit set. However, total yield did not correlate significantly with the other factors, likely due to large differences in fruit weight among cultivars.

4. Trellising system (2024)

For 2024, correlations among vegetative parameters were even stronger. The relationship between cultivar and shoot length was high (r = 0.743**, p < 0.01), confirming a stable genetic expression of vegetative vigor.

The number of shoots was positively correlated with the number of fruits (r = 0.700**, p < 0.01), and the relationship between buds and fruits was extremely strong (r = 0.903**, p < 0.01), suggesting efficient canopy management in the trellising system, which favors fruiting.

Fruit yield did not show significant correlations, indicating that fruit weight is more strongly influenced by technological factors and annual growing conditions than by the number of vegetative organs.

Tabel 1. The values of simple correlation coefficients between growth and fruiting characteristics in 17 analyzed blueberry varieties (*V. corymbosum* L.)

Growing system	Anul	Caracteristici analizate	Variety	Stems (No)	Shoots (cm)	Buds (No)	Fruits (No)	Fruit production (g)
Untrellising	2023	Variety	1					
		Stems (No)	,331*	1				
		Shoots (cm)	,525**	,515**	1			
		Buds (No)	0,113	,699**	,320*	1		
		Fruits (No)	0,044	,650**	0,253	,986**	1	
		Fruit production (g)	,305*	0,097	-0,024	0,170	0,182	1
	2024	Variety	1					
		Stems (No)	0,023	1				
		Shoots (cm)	,764**	0,025	1			
		Buds (No)	-0,051	,647**	0,089	1		
		Fruits (No)	-,310*	,351*	-0,064	,479**	1	
		Fruit production (g)	-0,163	0,239	-0,133	,316*	0,197	1
Trellising system	2023	Variety	1					
		Stems (No)	,426**	1				
		Shoots (cm)	,626**	,396**	1			
		Buds (No)	0,251	0,255	,378**	1	,979**	
		Fruits (No)	0,274	0,179	,384**	,979**	1	
		Fruit production (g)	0,214	-0,075	0,024	0,109	0,159	1
	2024	Variety	1					
		Stems (No)	,395**	1				
		Shoots (cm)	,743**	,378**	1			
		Buds (No)	0,205	,553**	0,097	1		
		Fruits (No)	,388**	,700**	0,260	,903**	1	
		Fruit production (g)	0,276	0,080	0,239	-0,090	-0,016	1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

● The general trend confirms that blueberry trellising stimulates both vegetative and reproductive growth, ensuring a balance between vigour and fertility. Trellising corrects the position of shoots within the blueberry canopy and thus conduct to influences upon the growth and fruiting processes; however, it requires material investments, more time and technical expertise.

- The differences among cultivars reflect the adaptability of genotypes to controlled bush architecture, whereas untrellised cultivars exhibit greater biological plasticity but with more pronounced year-to-year variation.
- The most stable and strongest correlations were observed between the number of buds and the number of fruits, regardless of training system or year, confirming the decisive role of flowering potential in determining production level.
- The trellising system promotes a closer relationship between vegetative parameters (stems, shoots, buds) and fruiting, indicating a more efficient use of plant resources.
- In the untrellised system, correlations are more variable, suggesting a greater influence of external factors and a less uniform structural organization of the bushes.
- Total yield (g/plant) is not directly dependent on the number of fruits, indicating cultivar-specific differences in fruit size and average fruit weight, considering that the plants are still very young and have not yet reached their maximum fruiting potential.

ACKNOWLEDGEMENTS

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EVALUATION OF THE QUALITATIVE CHARACTERISTICS OF SOME NEW BLUEBERRY VARIETIES AT USAMV OF BUCHAREST

Mihaela BUJOR[1], Valerica TUDOR[1], Adrian ASĂNICĂ[1], Dan POPESCU[1], Oana Vasilica HERA[2], Răzvan Ionuț TEODORESCU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing Pitești - Mărăcineni, 402 Mărului Street, Mărăcineni, Pitești, Argeș County, Romania

Corresponding author email: valericatudor@gmail.com

Abstract

Cultivation of blueberries is of great importance nowadays due to the high antioxidant content of the fruits and for a healthy diet. Given the high demand for fruits on the domestic and foreign market, the use of fruits in current consumption and in the processing industry, the highbush blueberry (Vaccinium corymbosum L.), has become a species of interest both globally and in Romania in recent years. Therefore, there are currently numerous breeding programs, carried out in most countries with advanced fruit growing, based on specific breeding objectives aimed at creating new valuable blueberry varieties. In this paper, 17 blueberry varieties (Vaccinium corymbosum L.) from the Experimental Field of the Faculty of Horticulture in Bucharest were analysed, within the blueberry variety collection established in 2018. The results presented are important for farmers, providing them with the information they need to cultivate such varieties according to market preferences. The analysed varieties can be included as gene sources in the blueberry breeding program carried out at UASVM Bucharest.

Key words: *Vaccinium corymbosum L., biometric characteristics, biochemical characteristics*

INTRODUCTION

The cultivated blueberry is one of the species of berries that are of great interest to both growers and consumers. Blueberry cultivation is of great importance nowadays due to the high antioxidant content of the fruit and for a healthy diet (Edger et al., 2022; Asanica et al., 2017).

The high demand for fruit on the domestic and foreign market combined with the use of blueberry fruits for fresh consumption and processed drive the highbush blueberry (*Vaccinium corymbosum* L.) to become a species of interest both worldwide and in Romania in the recent years. The highbush blueberry stands out for its highly appreciated fruits for fresh consumption, having two to four times larger fruits than those of the wild blueberry (*Vaccinium myrtillus* L.) (Howell et al., 2001).

Blueberries are rich in bioactive anthocyanins, with high levels of malvidin, which is associated with antioxidant benefits that contribute to reducing the risk of diabetes (Herrera-Balandrano et al., 2021).

Blueberries have received much attention and are recommended as one of the five healthy fruits by the Food and Agriculture Organization, due to their beneficial effects on health, based on their antioxidant, anticancer, anti-inflammatory, antiseptic, antiproliferative, anti-aging, astringent, neuroprotective, cardio, vision and kidney protective properties and because they are a good source of fibre, vitamins (B, C and K), folic acid, fatty acids, polyphenols (anthocyanins) and minerals, such as Ca, Fe, K, Mg and Mn (Kuang et al., 2022; Banerjee et al., 2020; Kumar et al., 2022; Hakkinen &Toronen, 2000; Okan et al., 2018).

In a study conducted by Ojog el al., 2024, consumer preferences were presented regarding some of the fruit quality indicators (size, color, firmness, juiciness, taste and aroma) for 30 blueberry varieties, of which 8 were Romanian varieties. The most appreciated blueberry varieties were ‘Brigitta’, ‘Pink Lemonade’ and ‘Putte’, with high scores for almost all indicators, and the Romanian variety that ranked well was ‘Delicia’, especially for juiciness, firmness, colour and size.

This demonstrates how cultivars with stable fruit quality traits, such as firmness, may be more suitable for the retail market and much preferred by consumers (Hera et al., 2023; Hera, 2024).
There are currently numerous breeding programs, carried out in most countries with advanced fruit growing, based on specific breeding objectives aimed at developing new valuable blueberry cultivars (Song & Hancock, 2011; Popescu et al., 2021).
In Romania, the genetic improvement of blueberry to obtain new, productive varieties with large, aromatic fruits, resistance to winter frost remains a main objective in research programs. Fruit quality is specific to each variety and can be influenced by the way in which they are influenced by environmental conditions, applied cultivation technology, harvesting, transportation, cold storage, fruit packaging (Hancock, 2006; Forney et al., 2012). Apart from the commercial aspect, which is decisive for fruits dedicated for fresh consumption, an important role is played by the taste, but also by the other characteristic features of the fruits (average weight of a fruit, fruit firmness, ripening grouped in clusters, colour, aroma, consistency, etc.) (Kloet, 1980).
Starting with 2021, the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, started a blueberry breeding program, in addition to the general breeding objectives established for blueberries, specific objectives were targeted, such as: earliness, large fruit, variability of fruit colour and decorative appearance. Sixteen hybrid combinations were realised, resulting in 636 hybrid fruits (Asanica et al., 2021; Popescu et al., 2021). Currently, over 700 blueberry hybrids are under evaluation in the breeding field of the Faculty of Horticulture. Finally, given the great influence of environmental conditions on fruit quality, it is recommended that breeder's sample and examine fruits from advanced selections after several weeks of storage before releasing the variety (Sater et al., 2021).

MATERIALS AND METHODS

In this paper, 17 blueberry varieties (*Vaccinium corymbosum* L.) were analysed from the Experimental Field of the Faculty of Horticulture (Figure 1) in Bucharest, within the blueberry variety collection (Figure 2) established in 2018.



Figure 1. Experimental field of the Faculty of Horticulture



Figure 2. Collection of blueberry varieties

To evaluate the 17 blueberry varieties studied, determinations were made on the following quantitative and qualitative characteristics (Cociu & Oprea, 1989):

Biometric characteristics:
- the average fruit weight (g) was determined by weighing a sample of 20 fruits from the 5 plants/varieties using the PS Partner technical balance (in grams), after the picking, depending on the ripening period of each variety, calculating the average weight;
- the fruit dimensions (mm) was appreciated considering the height and equatorial diameter of each fruit measured with a digital calliper (mm);
- the shape index calculated according to the formula: height/diameter of the fruit;
- the fruit firmness (kgf/cm^2) was determined on a sample of 5 fruits/plant/varieties at each picking time using the Turoni TR penetrometer.

Biochemical characteristics of the fruits:
- the soluble dry matter (SUS) content (Brix%) of the fruits was determined by the refractometric method on a sample of 20 fruits from the 5 plants/varieties, with the Milwaukee MA871 digital refractometer;

- the fructose content (%) and glucose content (%) were determined, by the same method and on the same sample, using the Milwaukee MA884 and MA873 refractometers;
- the pH of the fruits was determined with the IQ Scientific digital pH meter, on average samples analysed at each harvest;
- total dry matter content (%) (SUT) determined using the UN110 Memmert oven, at a temperature of 105°C for 24 hours. The method consisted of blending the fruits until a homogeneous paste was formed, followed by weighing the initial tare. The next step consisted of weighing the trays filled with approximately 5g of sample. For each variety, two analysis samples were carried out and introduced into the oven. After 24 hours, the trays with the dehydrated samples were weighed and the SUT was calculated according to the formula:

Total dry matter (TDS) (%) = 100 – Moisture (%)
U% = (cp-cpu)/(cp-cg)*100
where:
U% = moisture %;
cg = empty zero weight;
cpu = zero weight with sample after drying;
cp = zero weight with sample before drying.

- total polyphenol content (TPC) (mgGAE/ 100 g) was determined using the Folin-Ciocalteu method, with a methanol solution of plant material, obtained by vortexing 1 g of plant material in 10 mL of 70% methanol in a Vortex Mixer VX-200 Corning-Labnet system. Absorbance was measured using a UV-VIS Specord 210 Plus spectrophotometer (Analytik Jena, Jena, Germany) at a wavelength of 760 nm;
- total titratable acidity (malic acid/100 g) was determined using an extract of plant material, to which 25 ml of distilled water was added, the initial pH value was measured, and then titrated with 0.1N NaOH until the pH reached 8.1, using the TitroLine Easy automatic titrator (SI Analytics GmbH, Germany). TTA results were expressed in grams of citric acid per 100 grams of fresh fruit. Total titratable acidity was expressed as a percentage of malic acid. Results were calculated using the formula:

Titratable acidity = ml NaOH 0.1N * 100 * 0.0067/sample mass

- antioxidant activity (mg equal Trolox/100 g fresh sample) was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, similar to that described by Petre et al. (2022): 0.2 ml of extract are mixed with 2 ml of 0.2 mM DPPH solution in 100% methanol and incubated in the dark for 30 minutes, with homogenization. The absorbance of the samples is measured at a wavelength of 515 nm. The solution used as reference was 100% methanol.

Data interpretation

Statistical analysis was performed using IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were statistically evaluated by analysis of variance (ANOVA). Differences between cultivars were highlighted through Duncan's multiple test range ($p< 0.005$). Graphical representations were performed with Microsoft Office Excel 2007.

RESULTS AND DISCUSSIONS

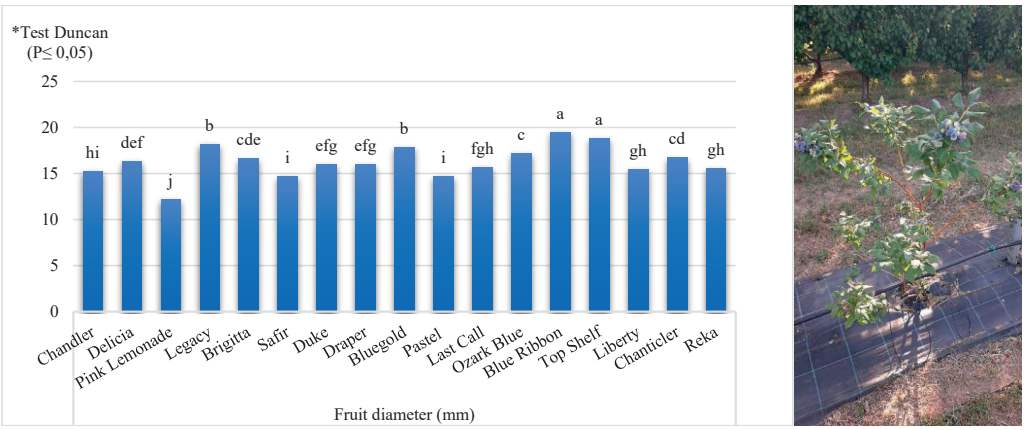
Biometric characteristics

The main biometric characteristics of blueberry fruit refer to the size, shape and texture of the pulp and are expressed by: fruit diameter, shape index, average weight and firmness.

Statistical analysis performed by univariate ANOVA demonstrated that there are significant differences between the studied varieties in terms of average fruit diameter ($p < 0.05$). The variety 'Pink Lemonade' has the smallest fruits (12.20 mm), significantly different from all other varieties.

The varieties 'Duke', 'Draper' and 'Delicia' have diameters around 16.0-16.3 mm, being in the median groups, with no significant differences between them.

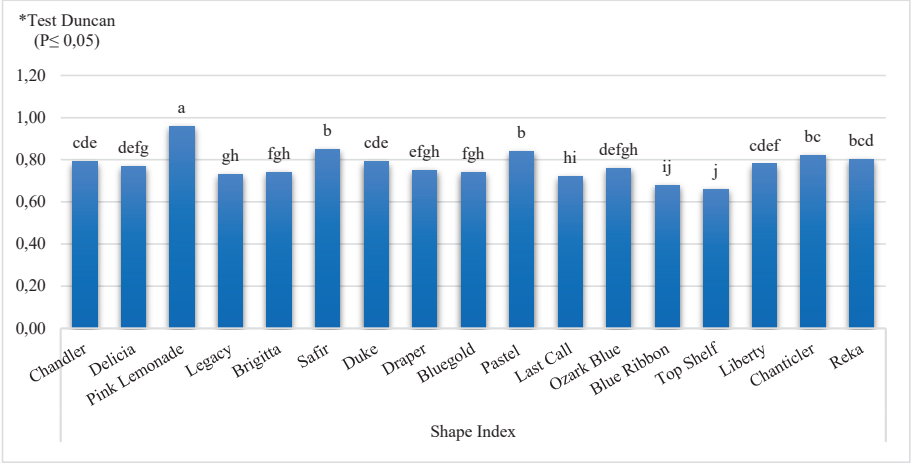
The highest diameter was measured in: 'Blue Ribbon' (19.5 mm), significantly larger than most of the analysed varieties (Figure 3), falling into the premium category, with a significantly higher price.



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 3. Variation in fruit diameter in cultivated blueberry

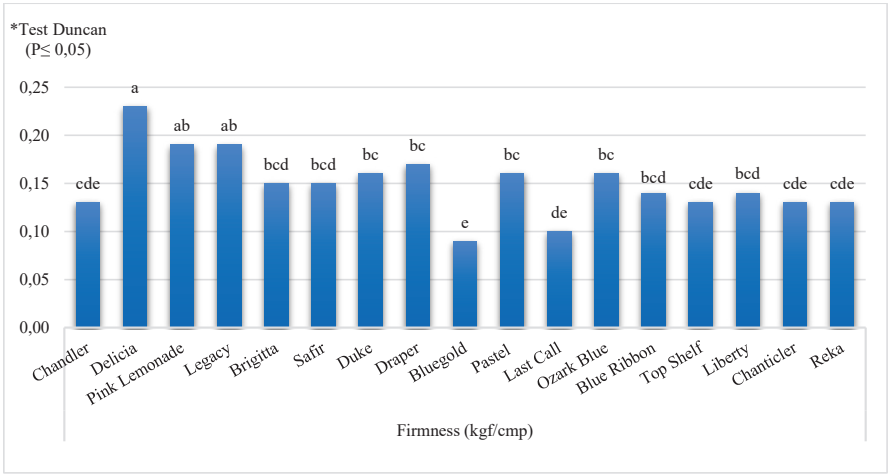
The shape index expresses the ratio between the longitudinal and transversal diameter of the fruit - higher values above 1 indicate more elongated fruits, values close to 1 indicate spherical fruits, and below 1 indicate flattened fruits. 'Top Shelf' (0.66) and 'Blue Ribbon' (0.68) are the most flattened, very significant differences compared to the rest of the varieties, recorded by the variety 'Pink Lemonade' (0.96) which stands out statistically from all the other varieties (Figure 4).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 4. Variation in fruit shape index in cultivated blueberry

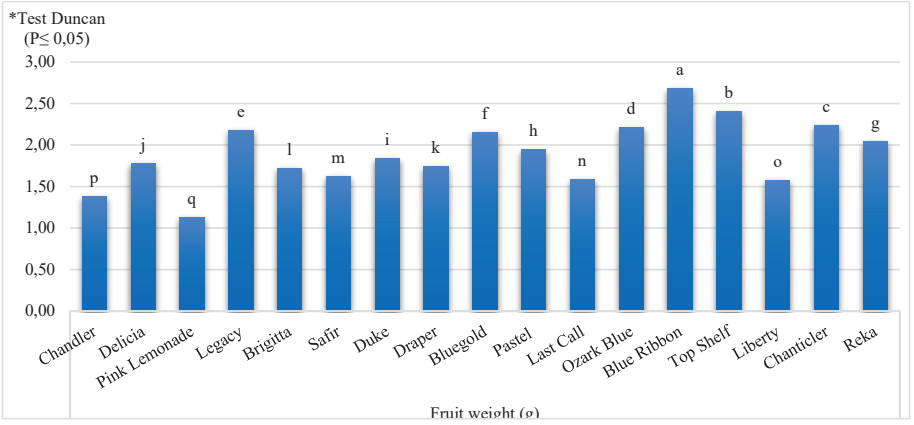
Varieties with high firmness are more suitable in this respect for mechanized harvesting and sale over longer distances. The processed data show significant differences between the studied varieties. The varieties 'Pink Lemonade', 'Legacy' and 'Delicia', with the index between 0.19-0.23, have a special resistance for the post-harvest segment (Figure 5), excellent for export and storage.



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 5. Variation in fruit firmness in cultivated blueberries

The average fruit weight differs significantly between varieties ($p < 0.05$), demonstrating the determining influence of the variety. 'Blue Ribbon', 'Top Shelf' and 'Chanticler' have a superior commercial value, recommended for export and the premium market. The highest average fruit weight was recorded by the variety 'Blue Ribbon' (2.68 g), and the lowest by the variety 'Pink Lemonade' (1.13 g), but it charms in terms of colour, a wonderful shade of dark, intense pink, extremely attractive and sweet taste (Figure 6).

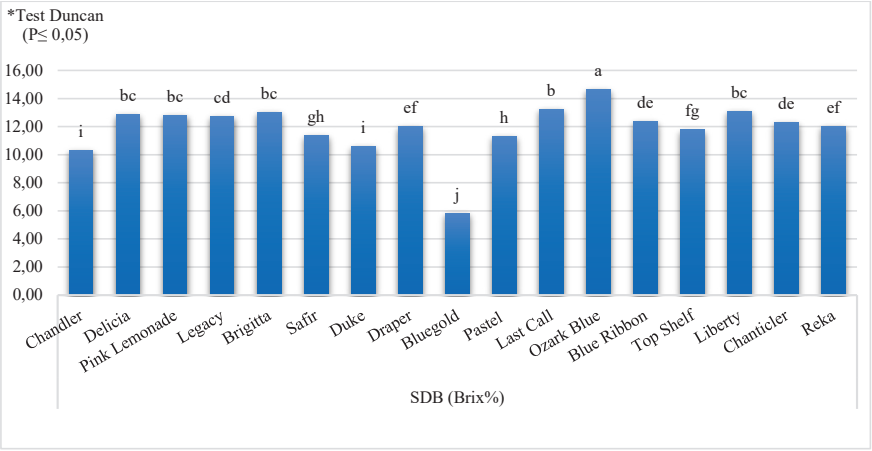


*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 6. Fruit weight variation in cultivated blueberry

Biochemical characteristics

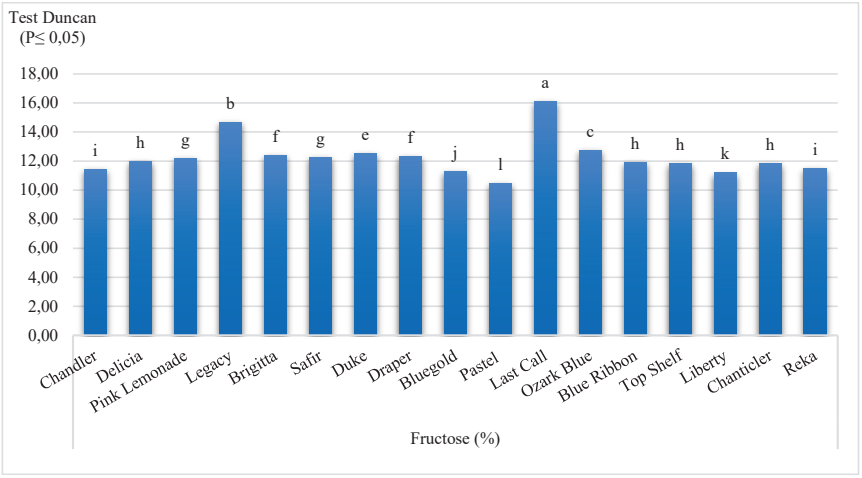
The soluble dry matter (SUS) content (Brix) level ranged between 5.83 ('Bluegold') and 14.66 ('Ozark Blue'), the sugar accumulated in the fruit being genetically determined, influenced by the intensity of photosynthesis and the physiological ripening specific to the variety. At the same time, the sweet taste attracts consumers, the varieties with values >12 °Brix ('Chanticler', 'Blue Ribbon', 'Legacy', 'Pink Lemonade', 'Delicia', 'Brigitta', 'Liberty', 'Last Call' and 'Ozark Blue') being recommended for fresh consumption (Figure 7).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 7. Variation in soluble dry matter content of cultivated blueberry fruits

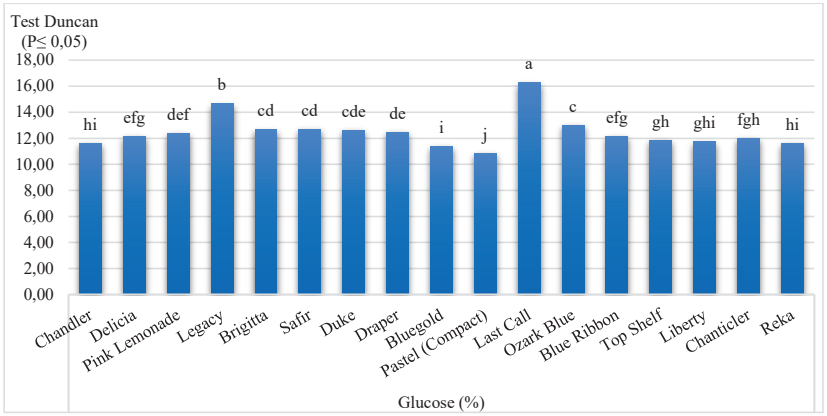
The analysis of the variable fructose content (%) shows that the variety significantly influences the accumulation of fructose in the fruit (Figure 8), with values ranging between 10.44% in the 'Pastel' variety, with minimal content and a less sweet taste, and 16.08 in the 'Last Call' variety, which is statistically distinguished by the highest degree of fructose accumulation.



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 8. Variation in fructose content of cultivated blueberry fruits

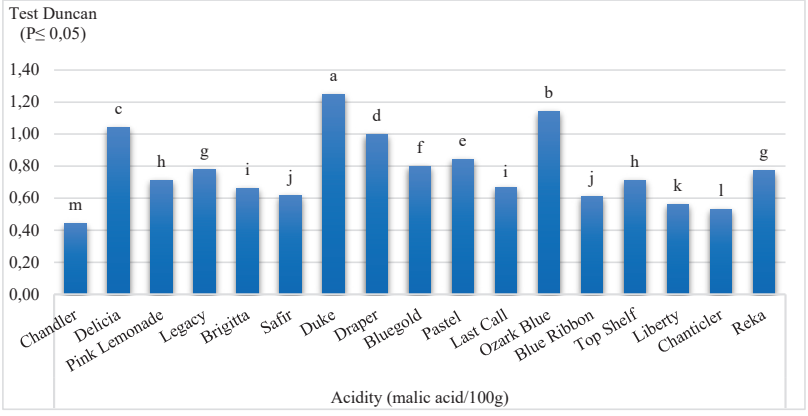
Concerning glucose content, the data obtained group the values in 10 classes, indicating consistent variations between varieties, with 'Last Call' having the highest content (16.26 mg/100 g), significantly different from most varieties (Figure 9).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 9. Variation in glucose content of cultivated blueberry fruits

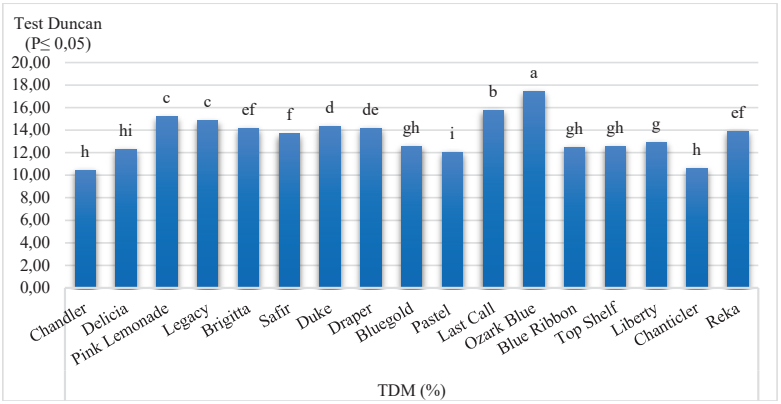
Acidity is a major indicator of freshness, oxidative stability, and shelf life. Varieties with high acidity are more resistant to post-harvest degradation and are more suitable for processing (juices, jams, freezing), while those with lower acidity are preferred by consumers looking for sweet fruits. The cultivar 'Duke' recorded the highest content of 1.25 malic acid/100 g, at statistically significant differences compared to the other cultivars, followed by the cultivar 'Ozark Blue' with 1.14 malic acid/100 g (Figure 10).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 10. Variation in titratable acidity of cultivated blueberry fruits

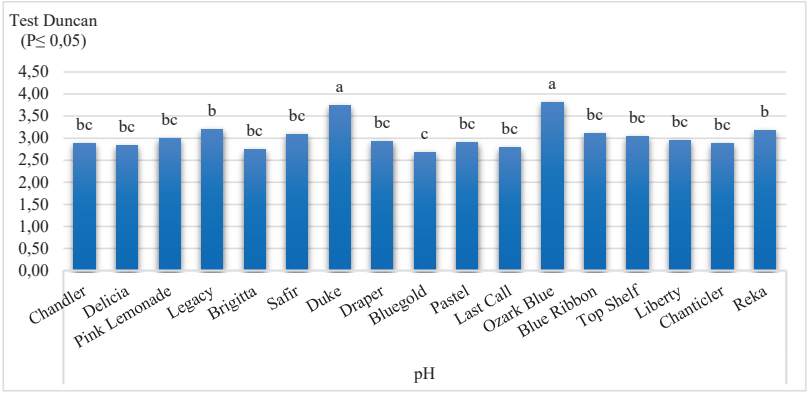
The soluble dry matter content - a cumulative indicator of sweetness and nutritional-energy potential, essential in breeding, was recorded by the varieties 'Ozark Blue' (17.43), 'Last Call' (15.76) and 'Pink Lemonade' (15.17) with the highest values (Figure 11).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 11. Variation in total dry matter content of fruits in cultivated blueberry

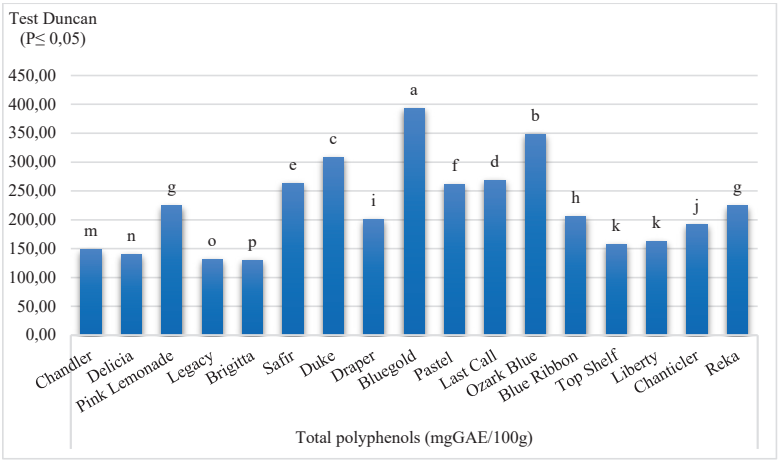
The analysis of the pH variable (the expression of acidity in the fruit pulp) indicates a moderate genetic variability, dividing the analysed varieties into 3 distinct groups, some with low pH (more acidic), moderate and high (less acidic). The first group includes the varieties 'Bluegold', 'Brigitta', 'Last Call', 'Delicia', 'Chanticler', 'Chandler', 'Pastel', 'Draper' with a content between 2.68-2.91. At moderate pH 2.95-3.20 are the varieties 'Liberty', 'Pink Lemonade', 'Top Shelf', 'Safir', 'Blue Ribbon', 'Reka', 'Legacy' which provide a sweet-sour balance, preferred for fresh consumption. The least acidic varieties are 'Duke' with pH 3.75 and 'Ozark Blue' with pH 3.81 (Figure 12).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 12. Variation in fruit pH in cultivated blueberries

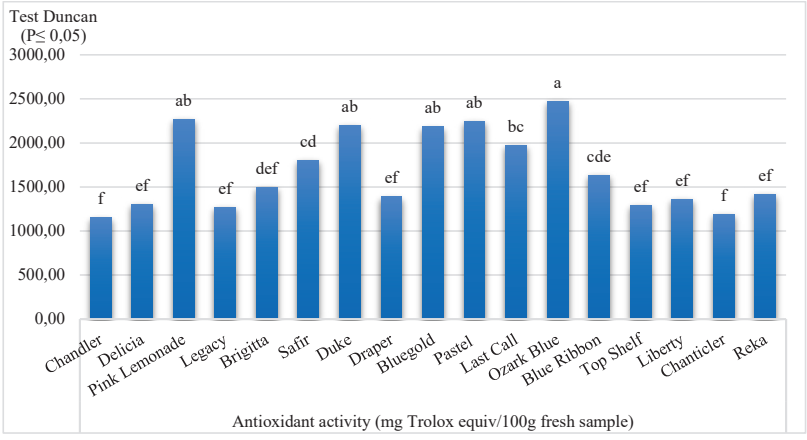
Polyphenols are one of the most important bioactive components of blueberries, contributing to their high antioxidant capacity, cardiovascular and neuroprotective protection, intense colour (through anthocyanins) and greater resistance to handling and storage. The varieties 'Brigitta' (129.77) and 'Legacy' (131.02) recorded the highest low values of total polyphenol content (mg GAE/100 g), they are suitable for fresh consumption (Figure 13). Recommended in functional nutrition, for supplements with therapeutic value, the varieties with content over 300 mg GAE/100 g are noted: 'Duke' (308.33), 'Ozark Blue' (347.6) and 'Bluegold' (393.02).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 13. Variation in total polyphenol content of cultivated blueberry fruits

Antioxidant capacity reflects the ability of bioactive compounds (especially polyphenols and anthocyanins) to neutralize free radicals - a key indicator of the therapeutic and nutritional potential of blueberries. The highest values were recorded in the varieties 'Ozark Blue' (2475.84), 'Pastel' (2248.42), 'Pink Lemonade' (2270.11), 'Duke' (2202.31) and 'Bluegold' (2181.88), which are classified as resources with high nutraceutical potential, recommended for functional food products and antioxidant supplements (Figure 14).



*Duncan test. Mean values with the same letter do not show significant differences (P≤0.05)

Figure 14. Variation in antioxidant capacity of cultivated blueberry fruits

CONCLUSIONS

The results are important for farmers, providing them the key supportive data for decision regarding choosing the proper blueberry varieties according to market preferences. The analysed varieties can be included as gene sources in the blueberry breeding program carried out at USAMV of Bucharest, towards obtaining new varieties with a large quantity of bioactive compounds in close correlation with the variety-specific phenotypic plasticity in response to gradual changes in environmental conditions from Romania.

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PRELIMINARY RESEARCH ON THE INFLUENCE OF CERTAIN AGROTECHNICAL MEASURES ON THE GROWTH OF THE 'ANNA SPÄTH' PLUM VARIETY UNDER THE CONDITIONS OF THE MOARA DOMNEASCĂ EXPERIMENTAL STATION, ILFOV COUNTY

Cristian Marian CALINITA[1, 2], Andreea Mihaela UDREA BRASLA[1, 2], Cătălin-Viorel OLTENACU[2], Mihai BOTU[1, 3]

[1]University of Craiova, Doctoral School of Engineering of Animal and Plant Resources, 13 A. I. Cuza Street, Craiova, Romania
[2]Research and Development Station for Fruit Tree Growing Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[3]University of Craiova, Faculty of Horticulture, 13 A. I. Cuza Street, Craiova, Romania

Corresponding author email: calinita.cristian@scdpbaneasa.ro

Abstract

The study (2022-2023) at Moara Domnească Experimental Base, affiliated with the Research and Development Station for Pomology Băneasa, Ilfov County, evaluated the effects of land shaping, localized irrigation, and two rootstocks on the growth of the 'Anna Späth' plum cultivar using a split-plot experimental design. Planting on ridged terrain did not significantly enhance growth parameters. In 2023, the trunk cross-sectional area was 1.23 cm² smaller per tree in ridged terrain than in flat terrain. Localized irrigation significantly improved growth. The trunk cross-sectional area increased by 0.86 cm² in the first year, 1.77 cm² in the second year, and 1.31 cm² on average. The number of shoots per tree rose by 2.77 (2022), 3.07 (2023), and 2.92 on average. Total shoot length increased by 183.5 cm (2022), 358.6 cm (2023), and 271.0 cm on average. The mean shoot length grew by 12.62 cm (2023) and 10.05 cm on average. No significant differences were observed between the two rootstocks. These results emphasize the benefits of localized irrigation in improving plum tree growth, while land shaping and rootstock selection had no notable impact.

Key words*: Prunus, land shaping, rootstock, irrigation, research.*

INTRODUCTION

The plum tree (*Prunus domestica*) represents the most extensively cultivated fruit species in Romania, owing to its high adaptability to diverse vegetation conditions and the multiple possibilities for fruit utilization. In intensive orchard systems, land preparation techniques play a crucial role in optimizing growth conditions and enhancing productivity. Studies have demonstrated that deep soil preparation prior to planting can provide substantial benefits during the initial fruit-bearing years. For instance, plowing to a depth of 55 cm has been shown to increase fruit yield by up to 322% in the second year compared to shallow plowing at 22 cm (Iancu, 2008).

In Romania, climatic conditions can significantly impact plum orchards, particularly in years characterized by insufficient or unevenly distributed precipitation. Under such circumstances, irrigation becomes a critical factor in ensuring optimal tree growth. Drip irrigation, widely employed in modern fruit-growing systems, has proven to be highly efficient in maintaining optimal soil moisture levels by enabling uniform water distribution and minimizing losses due to evaporation (Oltenacu et al., 2015). Research has indicated that this irrigation method significantly enhances tree growth parameters and is particularly beneficial for species sensitive to fluctuations in soil moisture (Septar & Stoli, 2019).

Furthermore, the rootstock onto which trees are grafted plays a pivotal role in influencing their ability to absorb water and nutrients from the soil. By selecting rootstocks adapted to local soil conditions, the water and nutrient uptake efficiency of moisture-sensitive cultivars, such as 'Anna Späth', can be significantly improved (Sumedrea et al., 2014).

To contribute to the optimization of growth and yield processes for the 'Anna Späth' plum cultivar under the specific conditions of the

Moara Domnească Experimental Base, Ilfov County, research was initiated in 2022. This study aims to assess the effects of various agrotechnical measures on tree development, with a particular focus on land preparation methods, irrigation strategies, and the selection of suitable rootstocks.

MATERIALS AND METHODS

The research was conducted on flat terrain, with a general slope of approximately 0.5-1%, on soil identified as reddish preluvosol, consisting of medium clay-loam.

Figure 1 shows the average annual precipitation recorded over a 33-year period at the meteorological station of the Institute of Meteorology in Bucharest, located approximately 1.5 km from the research site. The average was 589.08 mm, with annual values ranging from 327.2 mm in 2022 to 1053.7 mm in 2013.

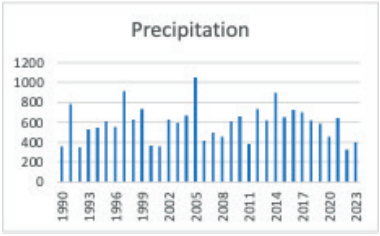


Figure 1. Annual Precipitation (mm)

To achieve the aforementioned objectives, the following factors were taken into consideration:

Factor A - Land Shaping, with the following treatments:

- a1 - Land Shaping in Ridges;
- a2 - Flat Land;

Factor B - Drip Irrigation Application, with the following treatments:

- b1 - Irrigated;
- b2 - Non-irrigated;

Factor C - Rootstock, with the following treatments:

- c1 - 'Mirobolan C5';
- c2 - 'Adaptabil'.

Thus, a 2×2×2 three-factorial experiment was established, with four replications and two trees measured for growth in each replication. The selected trees were isolated from neighboring ones to avoid competitive influences. Trunk thickness growth was measured by determining the diameter above the grafting point, and cross-sectional areas were calculated based on the measurements. Additional vegetative parameters such as number of shoots, total shoot length, and average shoot length were recorded annually after leaf fall in 2022 and 2023.

The plum rootstock selection in Romania includes native varieties adapted to local conditions, which provide advantages in terms of efficient water and nutrient supply, contributing to optimal plum growth. Among these, the 'Mirobolan' type rootstocks are the most widely used, representing approximately 85-90% of the total used in Romania. Recently certified rootstocks, such as 'Adaptabil' and 'Mirodad 1', have been developed to better address the climatic and soil challenges of specific regions (Botu et al., 2010).

The two rootstocks selected for this study exhibit different root system characteristics. 'Mirobolan C5' typically reaches rooting depths of 1-1.2 meters, while 'Adaptabil' has a shallower and more horizontally spread root system, with active absorption concentrated in the upper 60 cm of soil - an area particularly rich in nutrients and moisture. The choice of rootstock plays an essential role in determining tree vigor, the onset of fruiting, and also the quality of production - factors demonstrated in multiple studies on various Romanian and international plum cultivars (Zamfirescu et al., 2019; 2020; Iliescu et al., 2023).

In the variable climatic conditions of Romania, where precipitation is often insufficient or unevenly distributed, drip irrigation has become an essential practice in modern fruit growing. Its capacity to maintain consistent soil moisture, reduce evaporation losses, and improve water use efficiency has been demonstrated in multiple studies. For instance, Oltenacu et al. (2015) highlighted its role in enhancing vegetative growth and fruit quality, while Oltenacu N. and Oltenacu C.V. (2014) reported significant increases in plum production in the 'Anna Späth' variety under drip irrigation.

Figure 2 shows the application of irrigation between May 3 and September 28, 2022, with a total of 32 irrigation events, each providing 8 liters of water, resulting in a total input of 256 mm of water.

2022	Interval de 4 ore la fiecare irigare																													
LUNA/ZIUA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	TOTAL
APRILIE																																0
MAI			8			8				8			8				8								8			8				56
IUNIE																				8		8			8			8				24
IULIE	8				8			8			8			8				8			8			8					8			72
AUGUST		8			8				8			8										8			8					8		56
SEPTEMBRIE						8			8				8			8				8		8										40
OCTOMBRIE																																0
																												TOTAL			256

Figure 2. Drip Irrigation Application in 2022

Figure 3 shows the application of irrigation between April 28 and October 30, 2023, with a total of 35 irrigation events, each applying 10 liters of water, resulting in a total input of 348 mm of water.

2023	Interval de 5 ore la fiecare irigare																													
LUNA/ZIUA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	TOTAL
APRILIE																												8				0
MAI		10										20				10			20							10				10		60
IUNIE		10				10			10				10			10							10									60
IULIE											10							10							10			10				40
AUGUST				10				10			10				10			10				10			10				10			80
SEPTEMBRIE	10				10			10				10			10				10			10				10			10			90
OCTOMBRIE			10																													10
																														TOTAL		360

Figure 3. Drip Irrigation Application in 2023

Land shaping in ridges involved three successive plowing operations (November 2021, April 2022, and March 2023) that gradually formed ridges approximately 1.8 meters wide and 0.4 meters high. This system supports surface drainage, especially during periods of excessive precipitation, preventing water accumulation around the tree roots. Additionally, the ridging process displaced topsoil, considered to have superior physical, chemical, and biological conditions for root development.

This technique is supported by previous findings: Iancu et al. (1984) observed improved trunk growth in 'Agen 707' plum trees planted on ridged terrain compared to flat terrain on pseudogleized brown luvic soil in Zmeura, Argeș County. Similarly, Canarache (1986) highlighted the role of terrain modeling in facilitating surface drainage and improving plant health. Recent studies by Da Silva et al. (2024) further support the importance of soil hydraulic properties such as water retention $\theta(h)$ and hydraulic conductivity $K(h)$ for effective irrigation planning. Ridge formation improves root aeration, minimizes waterlogging risks, and reduces the incidence of diseases linked to excess soil moisture.

RESULTS AND DISCUSSIONS

Table 1 presents, for each of the two research years as well as their average, the differences in values between the levels of experimental factors regarding trunk thickness growth (mean/cm²). The data indicate that in the first year of research (2022), the cross-sectional area of the trunk measured in the ridge-shaped terrain variant (a1) was significantly larger by 0.70 cm²/tree compared to the flat terrain variant (a2), but only under irrigated conditions and for the 'Mirobolan C5' rootstock (b1c1).

In the same year, irrigation with 256 mm of water compared to the non-irrigated variant led to a highly significant increase of 0.86 cm²/tree across the entire experiment. The irrigation effect was significantly greater by 1.22 cm²/tree in the ridge-shaped terrain variant with the 'Mirobolan C5' rootstock and by 0.92 cm²/tree in the ridge-shaped terrain variant with the 'Adaptabil' rootstock.

In the second experimental year (2023), the data presented in Table 1 show that in the ridge-shaped terrain variant (a1), compared to the flat terrain variant (a2), trunk thickness growth was distinctly significantly lower by 1.23 cm²/tree. The differences between the two terrain modeling variants (a1 and a2) were significantly larger by 1.41 cm²/tree in the irrigated variant with the 'Mirobolan C5' rootstock and by 1.33 cm²/tree in the non-irrigated variant with the 'Adaptabil' rootstock.

On average over the two years, terrain modeling in ridges compared to flat terrain did not significantly influence trunk thickness growth for any of the experimental factors or their levels. However, irrigation (b1) compared to the non-irrigated variant (b2) resulted in a highly significant increase in trunk thickness of 1.31 cm²/tree. The differences were more pronounced, being distinctly significant at 1.47 cm²/tree for the 'Adaptabil' rootstock, and only significant at 1.21 cm²/tree for the 'Mirobolan C5' rootstock.

The data in Table 2 show that during the first experimental year (2022), the application of 256 mm of irrigation water, compared to the non-irrigated variant, led to an increase of 2.77 shoots per tree, with the difference being distinctly significant. The difference between the irrigated (b1) and non-irrigated (b2) variants regarding the number of shoots per tree was greater by 3.08 shoots per tree only in the variant where the land was shaped into ridges and for the 'Adaptabil' rootstock, with the differences being distinctly significant.

Table 1. The Influence of Experimental Factors and Their Levels on the Growth in Cross-Sectional Area of the Trunk of 'Anna Späth' Plum Trees in the First Two Years After Planting (2022-2023)

D	2022			2023			Avarage 2022-2023		
	E	LSD	F	E	LSD	F	E	LSD	F
a1-a2	0,27	0,208	n.s.	-1,23	0,002	**	-0,48	0,064	n.s.
b1-b2	0,86	0,000	***	1,77	0,000	***	1,31	0,000	***
c1-c2	0,04	0,843	n.s.	0,62	0,092	n.s.	0,33	0,196	n.s.
a1b1c1-a1b1c2	0,20	0,635	n.s.	0,32	0,439	n.s.	0,26	0,482	n.s.
a1b2c1-a1b2c2	-0,10	0,759	n.s.	1,15	0,137	n.s.	0,53	0,261	n.s.
a2b1c1-a2b1c2	-0,24	0,552	n.s.	0,36	0,706	n.s.	0,06	0,924	n.s.
a2b2c1-a2b2c2	0,30	0,603	n.s.	0,64	0,431	n.s.	0,47	0,440	n.s.
a1c1b1-a1c1b2	1,22	0,017	*	1,19	0,130	n.s.	1,21	0,041	*
a1c2b1-a1c2b2	0,92	0,036	*	2,02	0,002	**	1,47	0,003	**
a2c1b1-a2c1b2	0,38	0,405	n.s.	1,78	0,044	*	1,08	0,065	n.s.
a2c2b1-a2c2b2	0,91	0,124	n.s.	2,07	0,071	n.s.	1,49	0,068	n.s.
b1c1a1-b1c1a2	0,70	0,49	*	-1,41	0,015	*	-0,35	0,333	n.s.
b1c2a1-b1c2a2	0,26	0,601	n.s.	-1,37	0,172	n.s.	-0,56	0,395	n.s.
b2c1a1-b2c1a2	-0,14	0,783	n.s.	-0,82	0,391	n.s.	-0,48	0,439	n.s.
b2c2a1-b2c2a2	0,26	0,537	n.s.	-1,33	0,035	*	-0,54	0,239	n.s.

D - Notation of Differences Between the Levels of Experimental Factors (A, B, C)
E - Notation of Difference Values Between Experimental Factors
LSD – Least Significant Difference
F - Analytical Significance of the Presented Values
n.s - Not significant; * - Significant; ** - Distinctly significant; *** - Highly significan

For analysing the variance like in tabel above *, **, *** is need to add the LSA 5%, LSD1%, LSD0.1%

Table 2. The influence of experimental factors and their levels on the increase in the number of shoots per tree for the 'Anna Späth' cultivar during the first two years after planting (2022-2023)

D	2022			2023			Average 2022-2023		
	E	LSD	F	E	LSD	F	E	LSD	F
a1-a2	-0,52	0,558	n.s.	0,12	0,861	n.s.	-0,18	0,776	n.s.
b1-b2	2,77	0,004	**	3,07	0,000	***	2,92	0,000	***
c1-c2	-0,64	0,468	n.s.	1,00	0,151	n.s.	0,17	0,791	n.s.
a1b1c1-a1b1c2	-2,45	0,092	n.s.	2,44	0,127	n.s.	0,00	1,00	n.s.
a1b2c1-a1b2c2	-1,30	0,157	n.s.	0,00	1,00	n.s.	-0,66	0,455	n.s.
a2b1c1-a2b1c2	-0,88	0,716	n.s.	1,25	0,386	n.s.	0,19	0,892	n.s.
a2b2c1-a2b2c2	2,05	0,381	n.s.	0,31	0,828	n.s.	1,19	0,502	n.s.
a1c1b1-a1c1b2	1,93	0,199	n.s.	3,19	0,070	n.s.	2,56	0,073	n.s.
a1c2b1-a1c2b2	3,08	0,002	**	0,75	0,548	n.s.	1,91	0,020	*
a2c1b1-a2c1b2	1,58	0,473	n.s.	4,63	0,015	*	3,09	0,049	*
a2c2b1-a2c2b2	4,50	0,112	n.s.	3,69	0,034	*	4,09	0,054	n.s.
b1c1a1-b1c1a2	-1,58	0,511	n.s.	-0,38	0,813	n.s.	-0,97	0,526	n.s.
b1c2a1-b1c2a2	0,00	1,00	n.s.	-1,56	0,235	n.s.	-0,78	0,408	n.s.
b2c1a1-b2c1a2	-1,93	0,096	n.s.	1,06	0,442	n.s.	-0,43	0,661	n.s.
b2c2a1-b2c2a2	1,43	0,522	n.s.	1,38	0,347	n.s.	1,40	0,412	n.s.

D - Notation of Differences Between the Levels of Experimental Factors (A, B, C)
E - Notation of Difference Values Between Experimental Factors
LSD – Least Significant Difference
F - Analytical Significance of the Presented Values
n.s - Not significant; * - Significant; ** - Distinctly significant; *** - Highly significan

During the second experimental year (2023), the application of 348 mm of irrigation water compared to the non-irrigated variant resulted in an increase of 3.07 shoots per tree, with the difference being highly significant. This differentiation occurred only in the flat land variant, with the increase being significantly higher by 4.63 shoots per tree for the 'Mirobolan C5' rootstock and 3.69 shoots per tree for the 'Adaptabil' rootstock, with the difference also being significant.

On average over the two years (2022–2023), the application of 302 mm of irrigation water per year, compared to the non-irrigated variant, led to a highly significant increase of 2.92 shoots per tree. This difference was more pronounced in the 'Mirobolan C5' rootstock (3.09 shoots per tree) and lower in the 'Adaptabil' rootstock (1.91 shoots per tree), with the differences being significant in both cases.

The data in Table 3 shows that during the first year of experimentation (2022), the application of 256 mm of water through irrigation, compared to the non-irrigated variant, increased the total length of shoots per tree by 183.53 cm, the difference being highly significant. This differentiation was greater, at 263.13 cm/tree, in the flat-maintained soil and 164.7 cm/tree in the ridged soil.

Table 3. The influence of experimental factors and their levels on the total shoot length per tree for the 'Anna Späth' variety during the first two years after planting (2022-2023)

D	2022			2023			Media 2022-2023		
	E	LSD	F	E	LSD	F	E	LSD	F
a1-a2	23,36	0,505	n.s.	-115,73	0,132	n.s.	-46,20	0,340	n.s.
b1-b2	183,53	0,000	***	358,56	0,000	***	271,04	0,000	***
c1-c2	8,53	0,807	n.s.	126,46	0,102	n.s.	67,50	0,168	n.s.
a1b1c1-a1b1c2	20,31	0,837	n.s.	166,20	0,174	n.s.	93,25	0,345	n.s.
a1b2c1-a1b2c2	10,13	0,716	n.s.	47,75	0,584	n.s.	28,94	0,574	n.s.
a2b1c1-a2b1c2	-64,06	0,206	n.s.	148,44	0,516	n.s.	42,20	0,736	n.s.
a2b2c1-a2b2c2	67,70	0,459	n.s.	143,45	0,386	n.s.	105,56	0,356	n.s.
a1c1b1-a1c1b2	174,88	0,053	n.s.	368,90	0,010	*	271,88	0,010	*
a1c2b1-a1c2b2	164,70	0,048	*	250,44	0,034	*	207,56	0,028	*
a2c1b1-a2c1b2	131,38	0,054	n.s.	409,94	0,034	*	270,65	0,020	*
a2c2b1-a2c2b2	263,13	0,016	*	404,94	0,112	n.s.	334,03	0,047	*
b1c1a1-b1c1a2	51,83	0,489	n.s.	-155,75	0,180	n.s.	-51,97	0,479	n.s.
b1c2a1-b1c2a2	-32,56	0,691	n.s.	-173,50	0,456	n.s.	-103,03	0,469	n.s.
b2c1a1-b2c1a2	8,32	0,891	n.s.	-114,70	0,470	n.s.	-53,20	0,579	n.s.
b2c2a1-b2c2a2	65,88	0,370	n.s.	-18,98	0,841	n.s.	23,44	0,759	n.s.

D - Notation of Differences Between the Levels of Experimental Factors (A, B, C)
E - Notation of Difference Values Between Experimental Factors
LSD – Least Significant Difference
F - Analytical Significance of the Presented Values
n.s - Not significant; * - Significant; ** - Distinctly significant; *** - Highly significan

In the second year of experimentation (2023), the application of 348 mm of water through irrigation, compared to the non-irrigated variant, resulted in an increase in the total shoot length per tree by 358.56 cm, the difference being highly significant. This differentiation was more evident, at 409.94 cm/tree, in the flat-maintained soil variant with the 'Mirobolan C5' rootstock. In the ridged soil variant, irrigation, compared to the non-irrigated variant, also led to a significantly greater increase of 368.9 cm/tree for the 'Mirobolan C5' rootstock and 250.4 cm/tree for the 'Adaptabil' rootstock.

The data in Table 4 show that in the first year of the experiment (2022), the average shoot length in cm was not significantly influenced by any of the experimental factors or their levels. However, in the second year, the application of 348 mm of irrigation water, compared to the non-irrigated variant, resulted in a significantly greater increase of 12.62 cm in the average shoot length. This increase was significantly higher, by 15.51 cm/shoot, in the case of the ridge-shaped land modeling and the 'Adaptabil' rootstock.

Table 4. Influence of Experimental Factors and Their Levels on the Growth of the Average Length of a Shoot per Tree, in the 'Anna Späth' Cultivar, During the First 2 Years After Planting (2022-2023)

D	2022			2023			Averege 2022-2023		
	E	LSD	F	E	LSD	F	E	LSD	F
a1-a2	4,34	0,364	n.s.	-8,32	0,115	n.s.	-1,99	0,705	n.s.
b1-b2	7,47	0,124	n.s.	12,62	0,015	*	10,05	0,017	*
c1-c2	4,63	0,334	n.s.	7,11	0,123	n.s.	5,87	0,124	n.s.
a1b1c1-a1b1c2	13,60	0,147	n.s.	3,01	0,576	n.s.	8,30	,134	n.s.
a1b2c1-a1b2c2	11,48	0,198	n.s.	3,78	0,565	n.s.	7,63	0,285	n.s.
a2b1c1-a2b1c2	3,36	0,560	n.s.	7,81	0,600	n.s.	5,58	0,535	n.s.
a2b2c1-a2b2c2	-9,94	0,500	n.s.	13,85	0,192	n.s.	1,95	0,845	n.s.
a1c1b1-a1c1b2	7,29	0,426	n.s.	14,74	0,106	n.s.	11,01	0,144	n.s.
a1c2b1-a1c2b2	5,17	0,518	n.s.	15,51	0,037	*	10,34	0,122	n.s.
a2c1b1-a2c1b2	15,35	0,075	n.s.	7,10	0,639	n.s.	11,22	0,168	n.s.
a2c2b1-a2c2b2	2,05	0,880	n.s.	13,14	0,282	n.s.	7,60	0,497	n.s.
b1c1a1-b1c1a2	8,22	0,224	n.s.	-8,21	0,493	n.s.	0,00	0,817	n.s.
b1c2a1-b1c2a2	-2,02	0,803	n.s.	-3,42	0,761	n.s.	-2,72	0,730	n.s.
b2c1a1-b2c1a2	16,28	0,131	n.s.	-15,86	0,186	n.s.	0,21	0,916	n.s.
b2c2a1-b2c2a2	-5,14	0,706	n.s.	-5,79	0,445	n.s.	-5,46	0,599	n.s.

D - Notation of Differences Between the Levels of Experimental Factors (A, B, C)
E - Notation of Difference Values Between Experimental Factors
LSD – Least Significant Difference
F - Analytical Significance of the Presented Values
n.s - Not significant; * - Significant; ** - Distinctly significant; *** - Highly significan

On average over the two years, ridge-shaped land modeling compared to flat land did not cause a significant change in the average shoot length per tree for any of the experimental factors or their levels. Over the same period, no significant differences were recorded between the two rootstocks. However, during this period, at the level of the entire experiment, the application of irrigation in the irrigated variant, compared to the non-irrigated one, resulted in a significantly greater increase of 10.05 cm/tree

CONCLUSIONS

The influence of the experimental factors on the vegetative growth of the trees was evaluated over two consecutive years (2022-2023), using the following biometric indicators: trunk cross-sectional area, number of shoots per tree, total shoot length, and average shoot length.

Effect of Land Shaping (Factor A)
Planting trees on ridge-modified terrain (a1) compared to flat terrain (a2) did not result in significant differences in any of the measured growth parameters during the two experimental years or in the average values across the entire period. On the contrary, in 2023, a distinctly significant reduction in trunk cross-sectional area was observed in the ridge-modified variant, which was lower by 1.23 cm²/tree compared to the flat terrain variant. This suggests that, under the specific soil and climatic conditions of the experimental site, ridge shaping did not provide a vegetative growth advantage during the early establishment years of the orchard.

Effect of Drip Irrigation (Factor B)
The application of localized irrigation (b1) - with 256 mm of water in the first year and 348 mm in the second year after planting - had a consistent and statistically significant positive influence on all vegetative growth parameters when compared to the non-irrigated variant (b2). The study conducted by Venig and Stănică (2024) highlights that the application of irrigation and fertilization in the nursery significantly stimulates the photosynthetic rate in plum trees, contributing to the production of vigorous planting material. These results support the idea that the 'Anna Späth' cultivar can also benefit from such agrotechnical measures during the early growth phase, especially under current climatic conditions marked by drought.
A very significant increase in trunk cross-sectional area (cm²/tree):

- by 0.86 in the first experimental year;
- by 1.77 in the second year;
- and by 1.31 on average over the two years.

A distinctly significant increase in the number of shoots/tree in the first year by 2.77, and a very significant increase in the second year by 3.07, resulting in an average increase of 2.92 shoots/tree over the two years.
A very significant increase in total shoot length (cm/tree):

- by 183.5 cm in the first year;
- by 358.6 cm in the second year;
- and by 271.0 cm on average over the two years.

A significant increase in average shoot length (cm):

- by 12.62 cm in the second year;
- and by 10.05 cm on average across both years.

These results confirm the efficiency of localized irrigation in supporting vegetative development during the initial years after planting, under conditions of limited and variably distributed natural precipitation. Recent studies conducted at the Moara Domnească Station confirm that drip irrigation has a significantly positive effect on vegetative growth in plum cultivars, especially in 'Anna Späth', which shows increased sensitivity to water deficit (Udrea-Braslă et al., 2024).

Effect of Rootstock Type (Factor C)

No statistically significant differences were observed between the two rootstocks used 'Mirobolan C5' (c1) and 'Adaptabil' (c2)—in any of the analyzed growth characteristics, neither in the individual experimental years nor in the average values across the two-year period. This indicates a comparable vegetative performance under the experimental conditions, despite the differences in rooting depth and architecture between the two rootstocks. The study conducted by Pal et al. (2017) demonstrated that the choice of rootstock has a significant impact on tree vigor, fruiting branch formation, and productivity in high-density orchards. The low-vigor rootstock 'Gisela 5' induced earlier fruiting and nearly doubled the yield compared to the traditional Mahaleb rootstock. These results support the importance of careful rootstock selection in plum cultivation as well, in order to control vegetative growth and enhance the economic efficiency of the orchard.

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REACTION OF NEW INTRODUCED WALNUT CULTIVARS TO ANTHRACNOSE AND BACTERIOSIS IN BULGARIA

Angel DIMITROV[1, 2], Diyana ALEKSANDROVA[1, 2], Stefan GANDEV[1, 2]

[1]Fruit Growing Institute - Plovdiv, 12 Ostromila Street, Plovdiv, Bulgaria
[2]Agricultural Academy - Sofia, 30 Suhodolska Street, Sofia, Bulgaria

Corresponding author email: angel_dimitrov89@abv.bg

Abstract

The Walnut bacterial blight caused by Xanthomonas arboricola pv. juglandis and walnut anthracnose caused by the fungus Gnomonia leptostyla (sexual stage) and Marssonina juglandis (asexual stage) are the most economically significant walnut diseases in Bulgaria's climate. This study compared the susceptibility of newly introduced walnut cultivars Valmit, Valeris, Sebin, and Yalova 1 to these diseases with that of the prevalent Bulgarian cultivars Izvor 10 and Silistrenski. Observations for disease symptoms on leaves were conducted twice annually in the spring and autumn in a young walnut orchard at the Fruit Growing Institute – Plovdiv. The average percentage of walnut leaf spots showed that the Romanian cultivar Valmit (31.44%) was most affected. Another Rumanian cultivar Valeris was reported with the lowest percentage of infection to fungal disease with 22.30% infected leaves. From the observed cultivars, the most affected by bacterial blight was the Bulgarian one - Izvor 10 in degree 8.6%, which determinated it as a high susceptible to this disease. In our research the Turkish cultivar Yalova1 was reported as the lowest susceptible with 4.74% infected leaves.

Key words*: cultivars, Gnomonia leptostyla, infection, Juglans regia L., Xanthomonas arboricola pv. juglandis.*

INTRODUCTION

Walnut is a nut crop fruit belonging to the *Juglans* genus of the *Juglandaceae* family. Widely cultivated in different regions of the World, the walnut has an important role in human health due to its rich nutritional content (Carvalho et al., 2010). Walnut anthracnose, caused by the ascomycetous fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not. (anamorph *Marssonina juglandis* (Lib.) Magn.), poses a significant threat to walnut production worldwide (Yang et al., 2021). This disease is recognized as one of the most serious and widespread walnut afflictions, impacting nearly all walnut-growing regions. Its severity is closely linked to environmental conditions, with wet and rainy weather, particularly during spring, creating ideal conditions for rapid disease development and spread. The pathogen attacks various parts of the walnut tree, including leaves, twigs, shoots, and husks. Infected tissues develop irregular necrotic patches, often surrounded by distinctive small chlorotic halos. These lesions not only diminish the photosynthetic capacity of the tree but also directly impact fruit yield and quality (Belisario et al., 2008). Early infections can lead to fruit deformation, premature fruit drop, and reduced marketability. Furthermore, repeated defoliation, a common consequence of severe anthracnose infection, can have long-lasting detrimental effects on tree growth and vigor, occasionally leading to tree decline and even death. The economic impact of walnut anthracnose is substantial, with reported field losses reaching up to 50% in severely affected areas (Woeste et al., 2001). This underscores the critical need for effective disease management strategies to protect walnut orchards and ensure sustainable production (Yang et al., 2021). Bacterial pathogens are causing a lot of destructive crop diseases, which globally can have significant economic losses. There are over 25 genera and 200 species of bacteria that are pathogenic on plants (Sharma et al., 2022), many causing devastating outbreaks and substantial crop losses. Among damaging species belong to the genera *Xanthomonas* (Sharma et al., 2023). Host plant resistance plays a crucial role in the effective management of numerous plant diseases (Mehlenbacher, 1995), evaluating disease and pest resistance as a critical component of cultivar development. Deploying anthracnose-resistant cultivars offers a promising approach to disease management

and can be effectively integrated with other control strategies, such as targeted chemical applications, to minimize disease pressure (Berry, 1981). These two diseases target different parts of the walnut tree. *O. leptostyla* primarily infects leaves, petioles, and fruits, while *X. arboricola* pv. *juglandis* can infect a broader range of tissues, including leaves, catkins, female flowers, green branches, and nuts. Walnut blight, in particular, is known to significantly reduce both yield and the quality of harvested nuts (Arnaudov et al., 2014), impacting marketability and profitability. Research has demonstrated a strong cultivar-dependent response to *G. leptostyla* infection. Analysis of disease progression revealed significant variations in the number of leaf spots, the extent of fungal growth, and overall disease severity among different walnut cultivars (Dastjerdi, 2023). This variability in susceptibility underscores the importance of identifying and promoting the cultivation of resistant or tolerant cultivars as a key strategy for sustainable walnut production.

MATERIALS AND METHODS

In 2019-2021 period, monitoring the phytosanitary condition of walnut experimental orchards was conducted through route surveys in various phases of plant development. The surveyed plantations included two Turkish cultivars Yalova and Sebin, two cultivars from Romanian Valeris and Valmit and two Bulgarian cultivars Izvor 10 and Silistrenski which were grafted on common walnut (*Juglans regia* L.) rootstock.

The studied cultivars were monitored at Fruit Growing Institute in Plovdiv, the city has a humid subtropical climate with considerable humid continental influences. There are four distinct seasons with large temperature jumps between seasons. The development of the disease on the leaves is expressed by the attack index, which is calculated in percentages according to the formula of McKinney (1923). An infectious index was calculated for each cultivar, and the individual cultivars were classified according to their degree of susceptibility. The monitoring of walnut orchards was carried out two times per season, 100 leaves of each cultivar were randomly collected in May and September. The assessment of plant disease infections was conducted using the widely accepted six-point phyto-pathological scale (Table 1).

The data were statistically processed following Duncan's test (Steele & Torrie, 1980).

Table 1. Phyto-pathological scale

Degree of Susceptible	Degree of attack	
	Gnomonia leptostula Marssonina juglandis (%)	*Xanthomonas arboricola* pv. *juglandis* (%)
Immune	0	0
Resistant	1-5	to 1
Low susceptible	6-25	2-5
Moderately susceptibility	26-25	6- 10
Susceptible	36-50	11-20
Highly susceptibility	over 50	over 20

RESULTS AND DISCUSSIONS

The walnut cultivation area has become extensive, covering several regions in Bulgaria. The main diseases of walnut anthracnose and bacteriosis occur to varying degrees in different regions because of location, soil and cultivation conditions. Therefore, it is important to investigate the walnut level of susceptibility to anthracnose and bacterial blight in each cultivation region, especially newly introduced cultivars. On the other side, the effect of global climatic changes can lead to many adverse consequences, such as reduced yields, deteriorating fruit quality, susceptibility to diseases and pests, and reflect on the life of trees. Data analysis (Figure 1) showed the average rainfall and average temperature in the 2019-2021 period.

Moderate rainfall is observed throughout the 2019 year, with the wettest months being May and June, and the driest - August. The highest amount was recorded in June, with a significant peak of over 150 mm.

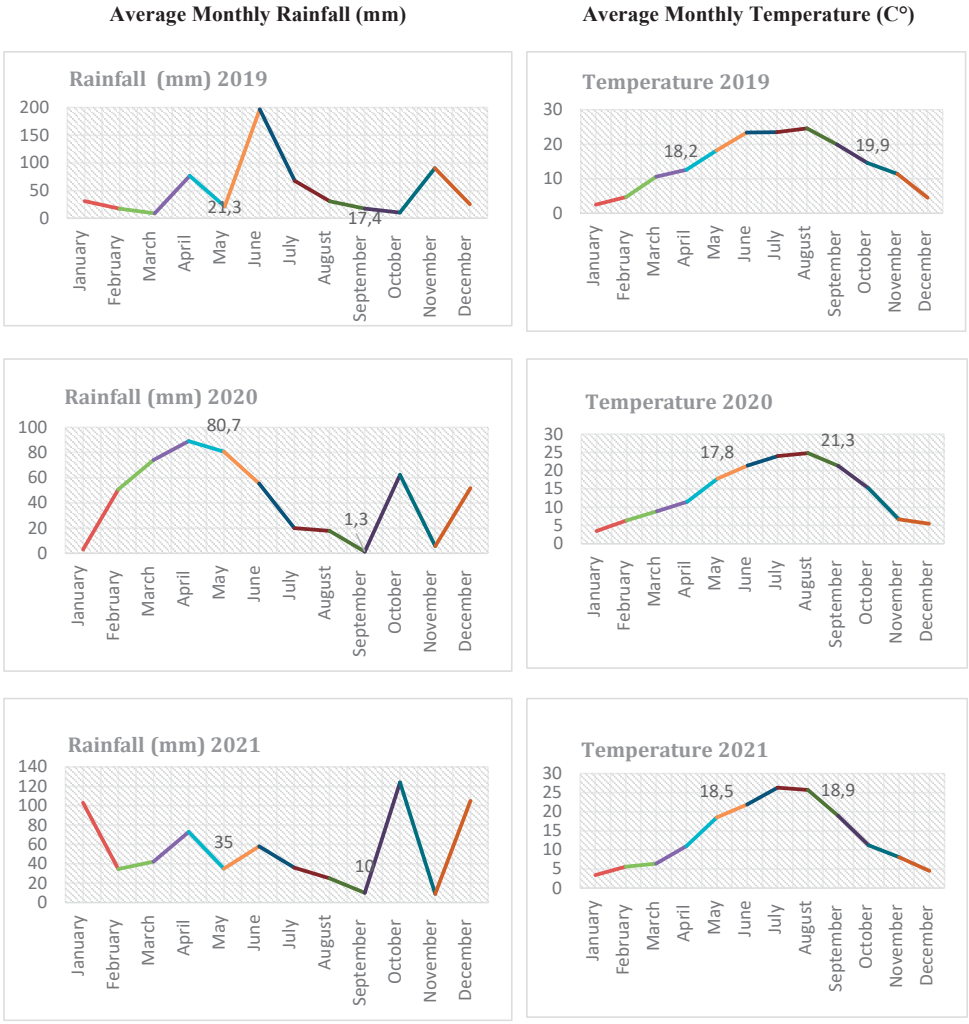


Figure 1. Climate data, registered for walnut vegetation in 2019-2021 for Plovdiv, Bulgaria

These climatic conditions create a favourable environment for the development of diseases, especially in the spring. 2020 was a year with variable rainfall, with the wettest months being May (21.3 mm), April, September (17.4 mm), and October. The driest month was January, followed by August.

The graph displays the monthly rainfall totals for 2021 in Plovdiv region. Overall, the year is marked by a relatively even distribution of rainfall. April has a significant increase in rainfall making it one of the wettest months. Rainfall in May was high but started to slightly decrease compared to April. The graph for 2021 shows the average monthly temperatures, the year follows a typical seasonal pattern of temperature change. The analysis data on climate shows that rainfall and temperature in three years were suitable for the development of the fungal disease (*Gnomonia leptostyla*) infection and secondary infections. In this period also accumulation of inoculum, which, when coupled with the frequent rainfall of autumn, leads to the development of the pathogen. From the end of May to the beginning of June, the first monitoring of symptoms of bacterial blight and walnut leaf spots on the leaves was done. Results of the study on the attack of leaves of walnut cultivars to anthracnose caused by the wintering form of

fungus *Gnomonia leptostyla* in the Plovdiv region (Table 2). The three-year study (2019-2021) on the degree of anthracnose infection in different walnut cultivars was analysed, and the Bulgarian cultivars Izvor 10 and Silistrenski work as a baseline for comparison.

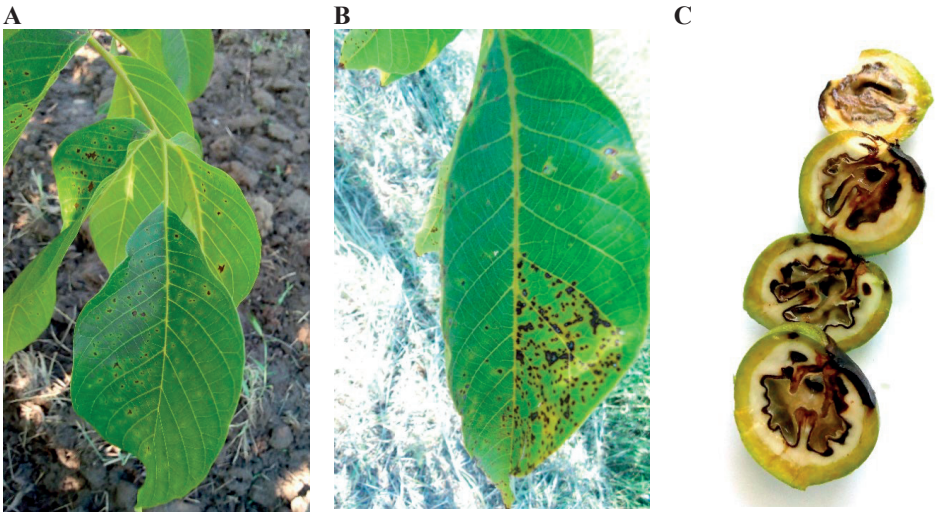


Figure 2. Symptoms of (A) bacterial blight (*Xanthomonas arboricola* pv. *juglandis*), (B) walnut leaf spot (*Gnomonia leptostyla*) and (C) apical necrosis

Table 2. Infection index on leaves of walnut cultivars

Cultivar	Leaves infection of *Gnomonia leytostyla and Marssonina juglandis* (%)								
	2019		Average	2020		Average	2021		Average
	I	II		I	II		I	II	
Yalova 1	18.33	31.43	17.02	3.40	30.37	9.29	5.80	61.14	33.47
Silistrenski	10.53	34.29	13.83	4.80	54.55	16.04	4.00	46.80	25.40
Valmit	18.66	52.63	22.49	0.60	25.63	6.71	8.20	96.00	52.10
Izvor 10	19.01	48.00	21.51	2.75	15.78	5.32	6.00	95.60	50.80
Sebin	20.00	25.14	16.28	2.20	23.45	6.96	5.20	98.00	51.60
Valeris	11.11	25.14	11.84	2.38	7.27	3.01	7.40	85.92	46.66

In comparison with Bulgarian cultivars in 2019, Izvor 10 (21.51%) showed similar levels of infection to Yalova 1 (17.02%) and Valmit (22.49%), but higher than Silistrenski (13.83%) and Valeris (11.84%). In 2020, Izvor 10 showed lower levels of infected leaves 5.32% than all other cultivars, except for Valeris, which had the lowest values with 3.01%. In 2021, Izvor 10 showed significantly higher levels of infestation 50.80% than all other cultivars, except for Sebin, which had similar values of 51.60%. The cultivar Izvor 10 showed the highest level of variable resistance to anthracnose over the years. Another Bulgarian cultivar Silistrenski in 2019 showed the lowest percentage of infected leaves. In 2020 Silistrensk showed higher levels of infected leaves than most cultivars. In 2021, Silistrenski showed lower levels of infestation than all studied cultivars. The comparison between Bulgarian cultivars Silistrenski showed better resistance to anthracnose compared to Izvor 10. Rumanian cultivar Valeris was measured with the lowest percentage of infected leaves in 2019 and 2020 the cultivar presented resistant stable to walnut leaf spots. The Turkish cultivar Yalova 1 showed lower resistance to anthracnose compared to Silistrenski and Valeris, but similar to Izvor 10. Atack of leaves of Shebin showed great variability in its resistance to anthracnose over the years. While it was with lowest infection index to the first report in May, it was highly attacked in September. The analysis of the data of the reaction of walnut cultivars can conclude that Silistrenski showed the best and most stable resistance to anthracnose among the studied cultivars, including in comparison with the other Bulgarian cultivar Izvor 10.

Table 3. Infection index on leaves of walnut cultivars

Cultivar	Leaves infection of *Xanthomonas arboricola* pv. *juglandis*								
	2019			2020			2021		
	I	II	average	I	II	average	I	II	average
Yalova 1	9.52	2.33	5.92	1.40	8.04	4.72	1.00	6.35	3.67
Silistrenski	14.29	11.18	12.73	5.16	7.57	6.37	1.40	4.90	3.15
Valmit	9.33	12.86	11.10	2.38	4.36	3.37	1.20	11.00	6.10
Izvor 10	14.29	17.60	15.94	0.90	5.05	2.97	2.20	12.00	7.10
Sebin	11.67	11.44	11.55	2.43	7.52	4.98	0.80	8.20	4.50
Valeris	7.27	20.00	13.64	2.87	5.56	4.21	1.20	7.55	4.38

Two times per year the reaction of walnut cultivars to bacterial pathogen *Xanthomonas arboricola* pv. *juglandis*.

The Turkish cultivar Yalova 1, showed good resistance to bacterial blight in each year of study. Levels of infection in Valmit were lower than those of Izvor 10 in most years but similar to or slightly higher than those of Silistrenski.

The cultivar Valmit performs better than Izvor 10 and is comparable to Silistrenski in terms of bacterial blight resistance.

Another Rumanian cultivar Valeris demonstrates the best resistance to bacterial blight among all the cultivars tested. The cultivar was significantly lower than those of all other cultivars, including the Bulgarian and Turkish ones.

The Bulgarian cultivar Izvor 10 was the most attacked cultivar to bacterial blight among those compared. Silistrenski showed better resistance than Izvor 10 but is more susceptible than Valmit and Valeris.

The average result (Table 4) presents the degree of susceptibility of six walnut cultivars to two diseases, a fungal infection caused by *Gnomonia leptostyla* or *Marssonina juglandis* and a bacterial disease caused by *Xanthomonas arboricola* pv. *juglandis*. The average percentage in tree studied years reflects the leaf area value affected by the respective pathogen. Cultivars which were low susceptibility to walnut leaf spots were Yalova 1 (23.36%) Valeris (22.30%).

Their average reaction showed that cultivars lowest are classified as "low susceptible" to both diseases. As moderately susceptible were distributed cultivars Valmit (31.45), Izvor 10 (29.47) and Sebin (27.65).

Non-significant differences between studied cultivars.

Table 4. Degree of susceptibility of walnut cultivars to *Gnomonia leytostyla* and *Xanthomonas arboricola* pv. *juglandis*

Cultivar	Leaves infection *Gnomonia leytostyla (%)*	Degree of susceptibility	*Xanthomonas arboricola* pv. *juglandis(%)*	Degree of susceptibility
Yalova 1	23.36 a	Low susceptible	4.77 a	Low susceptible
Silistrenski	23.36 a	Low susceptible	7.42 a	Moderately susceptibility
Valmit	31.45 a	Moderately susceptibility	6.85 a	Moderately susceptibility
Izvor 10	29.47 a	Moderately susceptibility	8.67 a	Moderately susceptibility
Sebin	27.65 a	Moderately susceptibility	7.01 a	Moderately susceptibility
Valeris	22.30 a	Low susceptible	7.40 a	Moderately susceptibility

The cultivar Yalova 1 showed the lowest susceptibility to bacterial blight (4.77%), making it the most resistant of the cultivars tested. Other walnut cultivars Silistrenski, Valmit, Izvor 10, Sebin and Valeris were distributed as moderately susceptible and showed similar susceptibility levels, ranging from 6.85% to 8.67%. There were statistically non-significant differences in susceptibility to the bacterial blight. Similar results were published by other authors in which studies have shown that Bulgarian local walnut cultivars are

generally susceptible to bacterial blight and exhibit slight susceptibility to walnut blight (Gandev et al., 2014; Blagoeva, 2022). In organic-grown orchards incidence of walnut leaf spots was reported Sebin was more susceptible from Izvor 10 (Zhelev et al., 2016) in this study both cultivars reacted almost similarly.

CONCLUSIONS

Global climate change, marked by rising temperatures, shifts in precipitation patterns, and an increasing frequency of extreme weather events, exerts a complex and multifaceted influence on the physiological processes, growth, and development of all fruit trees, including walnuts. These adverse factors can disrupt photosynthesis, and impair nutrient uptake ultimately impacting the productivity and longevity of these trees. In the comparative analysis, it is clear that Yalova 1 is the most resistant cultivar to anthracnose and bacteriosis compared to other studied walnut cultivars. The cultivars Valeris and Silistrenski were reported with the lowest percentage of infection and classified as having a low susceptibility to anthracnose. This makes them a good choice for areas where these diseases are prevalent. Other Rumanian and Turkish cultivars can be grown in Bulgaria conditionals with additional plant protection programs.

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RESEARCH ON THE INFLUENCE OF THE SOIL MAINTENANCE SYSTEM ON THE QUALITY OF CURRANT FRUITS

Maria Marinela DRAGNEA[1], Cătălin-Viorel OLTENACU[2], Mihai GÎDEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research and Development Station for Fruit Growing Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: maria.banca95@yahoo.com

Abstract

The research carried out follows the influence of the soil maintenance system on the quality of fruits from currant plantations in the climate and soil conditions of the northwestern Romanian Plain. Weed control in orchards by manual weeding and the use of mechanical means requires a lot of labor and the allocation of significant resources. A wide range of pre-emergent and post-emergent herbicides are used for chemical weed control, which are very effective in orchards of fruit trees. The problem of the use of herbicides in fruit growing must be considered not only from the perspective of the immediate effect on the plantation (total or partial elimination of weeds), but also from the point of view of the quality of the fruits, determining the specific biochemical parameters.

Key words*: maintenance system, plantation of fruit trees, fruit quality.*

INTRODUCTION

The exploitation of fruit ecosystems must be done with maximum efficiency, without aggressions and ecological vulnerabilities harmful to humans, animals and the environment. On the horizon of the 2050s, the expansion of the use of herbicides is expected primarily due to the acute lack of labor and the need to increase economic efficiency. That is why the problem of using herbicides in fruit growing must be seen not only from the perspective of the immediate or subsequent effect on the orchard, on the fruits, but also from the point of view of the quality of the environment in the entire fruit growing ecosystem. All categories of herbicides are phytotoxic for trees and can cause herbicide residues in fruits when the application instructions are not strictly followed, especially the dose and concentration of the herbicide solution, the time of application, the equipment (nozzles) used, the type of soil on which the plantation is located, the spectrum of weeds to be combated and the types of herbicides used, the climatic conditions and many other factors.
In Figure 1 we present the factors that influence the quality of the fruits and the critical points of risk of the pollution of the trees, the fruits, the environment in the orchard, namely: the pedoclimatic factors: the relief, the soil, the light, the temperature, the humidity; physiological factors: leaf surface, photosynthesis, transpiration, respiration; biological culture factors: variety and rootstock; technological: density, management form, cutting, rationing, fertilization, irrigation, weed control, phytosanitary protection; harvesting and storage factors: time of harvesting, method of harvesting, handling and storage conditions.



Figure 1. The factors that influence the quality of fruits (edited by M. Berca)

MATERIALS AND METHODS

Regarding the soil maintenance system, the variants that were studied in the experiment were: the black field and the grass strips.

The black field

It consists in keeping the soil loose and free of weeds through a permanent mobilization of it through works performed with mechanical or manual means. Analyzed over short periods of time, the black field increases the degree of loosening of the soil and improves the aeration regime on the worked area and the speed of water penetration. Also, by destroying weeds, it conserves water better than strip irrigation (Sumedrea et al., 2002). Tillage causes an increase in biological activity, probably through the release of polysaccharide-type substances fixed between the glomerular aggregates, inaccessible to the activity of microorganisms, but also following the improvement, at least in the short term, of some physical conditions of the soil, especially aeration and water.

Grass strips

By fertilizing, the structure of the soil improves due to the channels that remain in the soil after the death of the grass roots, as well as due to the change in the size of the pores as a result of the pressure exerted by the roots on the soil particles and microaggregates. Also, the grass extracting water from the soil causes a contraction and respectively a cracking of the soil which contributes to increasing its degree of loosening. Rain causes an increase in the number of earthworms in the soil and favors their activity, contributing in this way to the increase in the soil loosening process.

Study area

The study was carried out at the Moara Domnească Experimental Base of the Research and Development Station for Fruit Growing, Băneasa, located N-E of Bucharest in Afumați, Ilfov County, part of Vlasiei Plain, a subunit of the Roman Plain (44°50' Northern latitude and 26°24' Eastern longitude and 70 m above the sea level), on five currant varieties: Jonkheer Van Tets (red currant), Rovada (red currant), Tisel (black currant), Abanos (black currant) and Detvan (red currant).

Jonkheer Van Tets (Figure 2) is perfect for smaller or larger gardens, being able to be planted both in the open field and in planters. It is extremely resistant to frost (-20 degrees). It is a very productive variety with medium-sized fruit, spherical in shape, red-glossy when ripe. The fruits are juicy, with a particularly good, sweet-sour taste, they grow in the form of long bunches.



Figure 2. Jonkheer Van Tets variety

Rovada (Figure 3) is a variety with long bunches. Forms a bush with vigorous growth. Loves sunny or semi-shaded places. The berries are large, spherical and red in long clusters. The pulp has a pleasant sour taste, which is maintained even after baking.



Figure 3. Rovada variety

Tisel is a variety with a fairly airy spherical bush with erect stems. It is one of the most productive varieties and quantities of over 15 tons of fruit/ha can be obtained.

Abanos (Figure 4) forms a vigorous bush with stems of medium thickness. The berries are large and intense black and shiny. The pulp has a

pleasant sour taste. The fruits are recommended to be consumed processed in juice, jam, jelly or wine.



Figure 4. Abanos variety

Detvan (Figure 5) is a new variety obtained in Slovakia recognized for its resistance to diseases and frost. The plant forms a vigorous bush, vertical position, with fast growth, height can exceed 1.5 m, slightly compact with thick branches and large leaves.



Figure 5. Detvan variety

RESULTS AND DISCUSSIONS

In order to elucidate how soil maintenance systems can influence the quality of fruit production, biochemical determinations were made regarding their influence on fruit carbohydrate content and acidity.

Also, the degree of fruit firmness was studied in the five currant varieties, depending on the soil maintenance technology in the plantation.

The highest carbohydrate content was recorded in the Tisel variety with 19.5% Brix, in the black field maintenance system, and the lowest concentration was recorded in the Rovada variety with 10.1% Brix in the grassy strip maintenance system.

Regarding the pH value, it varied in the range of 2.79-2.94 for the black field system and 2.78-2.90 for the grass strip system.

The lowest value in terms of citric acid concentration was recorded in the Rovada variety, both in the black field system and in the soil maintenance variant with grassy strips.

With regard to fruit firmness, it can be seen that the production obtained in the system with grassy strips shows slightly higher values in all varieties, except for the Rovada variety.

The highest value of firmness was recorded in the Abanos variety (0.52 kgf/cm^2), and the lowest in the Jonkheer Van Tets variety (0.15 kgf/cm^2).

The average results recorded in 2024 are presented in Tables 1 and 2, but also in Figures 6, 7 and 8.

Table 1. The influence of the maintenance system - black field on the main physical and biochemical characteristics of currant fruits, at harvest, 2024

Variety / Parameters	Tisel	Abanos	Detvan	Rovada	Jonkheer van Tets
Carbohydrates (% Brix)	19.5	17.1	11.0	10.3	11.8
pH	2.85	2.88	2.94	2.85	2.79
Citric acid (%)	2.48	1.65	2.41	1.00	1.72
Firmness (kgf/cm^2)	0.45	0.44	0.26	0.27	0.15

Table 2. The influence of the maintenance system - grassy strips on the main physical and biochemical characteristics of currant fruits, at harvest, 2024

Variety / Parameters	Tisel	Abanos	Detvan	Rovada	Jonkheer van Tets
Carbohydrates (% Brix)	18.7	17.2	10.4	10.1	10.7
pH	2.78	2.85	2.90	2.78	2.81
Citric acid (%)	2.62	1.78	2.47	1.27	1.78
Firmness (kgf/cm^2)	0.49	0.52	0.31	0.26	0.18

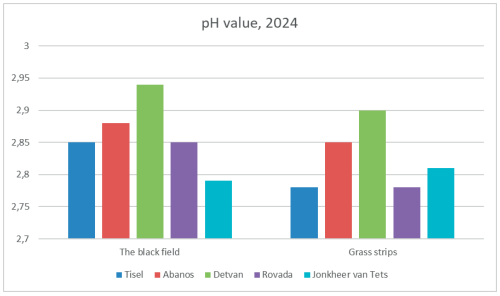


Figure 6. Comparative pH values recorded by currant varieties in 2024

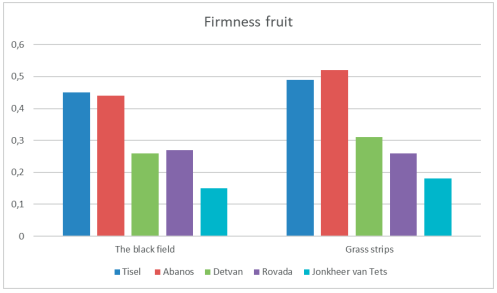


Figure 7. Comparative firmness values recorded by currant varieties in 2024

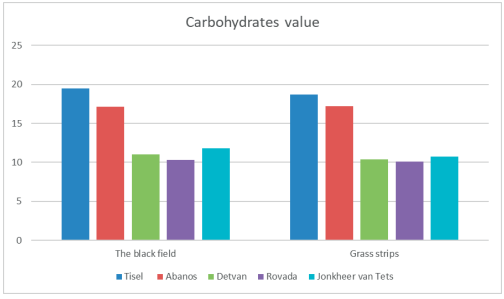


Figure 8. Comparative carbohydrates values recorded by currant varieties in 2024

CONCLUSIONS

The research carried out looked at the influence of the soil maintenance system on the quality of the fruit from fruit bushes (currant) plantations in the climate and soil conditions of the northwestern Romanian Plain.

The values recorded for the determined biochemical and physical parameters are relatively close between the two soil maintenance systems. The influence of the genetic factor, respectively of the variety, is more significant in relation to the influence of the soil maintenance system, this being valid for the experimental data recorded in 2024.

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EVAPOTRANSPIRATION AND INTERACTIVE EFFECTS OF IRRIGATION AND FERTIGATION ON WHITE STRAWBERRY FRUITS

Elena GRANCHAROVA[1], Blagoj ELENOV[1], Petya IVANOVA[2], Emilija ELENOVA[1], Georgi KOSTADINOV[1]

[1]Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", 7 Shosse Bankya Str., Sofia, Bulgaria
[2]Institute of Food Preservation and Quality, 154 Vasil Aprilov Blvd, Plovdiv, Bulgaria

Corresponding author email: eveha@abv.bg

Abstract

This research presents the crop evapotranspiration and investigates the effects of drip irrigation and fertigation on white strawberry fruits (diameter and number of fruits per plant). A two-factor experiment was conducted during 2023 and 2024 in an unheated greenhouse in the Chelopechene experimental field, Sofia, Bulgaria with drip irrigated and fertigated strawberry cultivar (Fragaria × ananassa 'Snow White'). The irrigation and the fertilization factors were applied in two rates: I1 - 75% (ETc) I2 - 50% (ETc), F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 – suboptimal fertilization - 75% (F1). Total strawberry evapotranspiration was 380.99 mm for 2023 season and 416.34 mm for 2024 season. The results showed that the highest mean fruit diameter (d) was obtained from I1F2 treatment (22.23 mm) in 2023 and 21.55 mm in 2024 from I1F1. There was a significant reduction (12%) in fruit diameter (d) in the second growing year (2024) in the most unfavourable growing treatment (I2F2). The fruit weight reduction from I2F2 treatment was 31% in 2024. The highest mean number of fruits per plant was obtained from I1F1 treatment – 60.97 pcs in 2023 - 2024. As opposed to fruit sizes and fruit weight the fruits number per plant increased. The fruits number per plant increasing from I1F1 treatment was 84% in 2024.

Key words*: white strawberry, irrigation, fertigation, fruit diameter, fruit weight.*

INTRODUCTION

The evapotranspiration, using current climatic data, estimate the crop water requirements and might serve to achieve better irrigation water productivity. The evapotranspiration estimation plays an important role in strawberry water requirements determination as a drought sensitive plant (Celiktopuz, 2023). The dynamics of change in current climate data due to climate changes draws researcher`s attention on strawberry evapotranspiration observations (Janssens et al., 2024; Jo et al., 2021; Menzel, 2021; Santosh, 2021).

It is essential to find the balance between irrigation and fertigation to achieve high yield and good quality while at the same time reducing the risk of soil nitrate nitrogen leaching (Bhagwat et al., 2023; Kulikov et al., 2020; Li et al., 2024; Richa & Sanjeev, 2022; Webler et al., 2023)

Strawberries with white fruits have long history of cultivation no shorter then red varieties. White strawberry has cultivated for hundreds of years in Chile and has grown in two botanical forms - wild *Fragaria chiloensis* ssp. *chiloensis* f. *patagonica* and cultivated *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* (Grez et al., 2020). It was brought to Europe in the 18th century. 'Snow White' cultivar has been selected in 2010 out from *Fragaria x ananassa* 'Weisse Ananas' and *Fragaria chiloensis* f. *chiloensis* (Olbricht et al., 2013). White strawberries are distinguished by their white or pale pink flesh and unique pineapple-like flavor.

This research presents the crop evapotranspiration and investigates the effects of drip irrigation and fertigation on white strawberry fruits (diameter and number of fruits per plant).

MATERIALS AND METHODS

A two years two-factor experiment was conducted on drip irrigated strawberry plants in a tunnel greenhouse in 2023 and 2024 in the Chelopechene experimental field (latitude 42°44′22.8″N, longitude 23°28′3.7″E and altitude 550 m above sea level) of the Institute of Soil Science, Agrotechnologies and Plant

Protection "Nikola Poushkarov" in Sofia, Bulgaria. Sofia field falls into temperate continental climate subzone. The greenhouse was unheated with area 420 m^2 (7.9 m x 53 m) covered with a five-layer UV+EVA+IR+AD+dif -150 µm polyethylene film. The soil could be defined as moderate to strong water-permeable with an average filtration capacity. The soil was *Chromic Luvisol* with bulk density 1.47 g cm^{-3}, field capacity 22% and wilting point 10% for 0-50 cm layer. Pre-planting physico-chemical characteristics of the soil (0-20 cm) at the experimental plot were pH 6.5, organic carbon 2.87% and available nitrogen (N) 19.30 mg kg^{-1}, phosphorus (P_2O_5) - 4.35 mg kg^{-1} and potassium (K_2O) - 2.79 mg kg^{-1}. To further reduce water losses, mulching with silver-black UV polyethylene mulch with a thickness of 30 µm was applied.

The object of the study was white strawberry cultivar (*Fragaria* × *ananassa* 'Snow White'). The experimental treatments were arranged according to the method of long plots with three replications. Each plot has 23.2 m^2 area and consisted of twin rows of strawberries. Healthy bare-root frigo plants were planted in scheme of 90 + 30/30 cm on 22 March 2023. According to the white strawberries cultivation technology in each of the experimental plots were provided the appropriate amount of red fruit plants (4: 1 ratio) to ensure better pollination. The irrigation factor was applied in two rates: I1 - deficit irrigation - 75% (ETc); I2 - deficit irrigation - 50% (ETc). The fertilization factor was applied in two rates: F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 - suboptimal fertilization - 75% (F1) - $N_{6.07}P_{9.57}K_{11.94}$. Optimal fertilization was developed according to Haifa nutrition recommendations (Haifa Group, 2021) as follows: Haifa MAP- 25-45 kg ha^{-1} , Multi K – 80 kg ha^{-1} , Haifa MKP - 25 kg ha^{-1} , Haifa Cal - 30 kg ha^{-1} , Maguisal - 10 kg ha^{-1} , Poly-feed - 25-40 kg ha^{-1}. Five treatments were tested: control treatment I0F0: 100% (ETc) - full irrigation and without fertigation; I1F1; I1F2; I2F1; I2F2. Irrigation was applied trough drip system include NMC Junior controller for precise irrigation rate application, FertiKit Nutrigation system for precise fertigation rate application, pressure-compensated pipelines UniRam AS with 14.6 mm inside diameter, 1.2 mm wall thickness, built-in trough 20 cm drippers and flow rate 1.6 l h^{-1}.

The microclimate data (air temperature, relative humidity, solar radiation, sunshine duration and wind speed) in the greenhouse was measured at every 30 min using an automatic meteorological station located in the centre of experimental area and recorded in data logger (HOBO USB Micro Data Logger, USA). FAO Penman-Monteith Equation (Allen et al., 2006a) was used for determining daily reference evapotranspiration and irrigation scheduling. Crop coefficient was 0.30, 0.80 and 0.70 respectively in initial, middle and end growing stage (Allen et al., 2006b). Fruit mass was determined through weighing with a electronic precision balance Vedia FR-H (±0.01 g). Fruit width and length was measured with a digital calliper (±0.01 mm). All the observations were carried out of 5 consecutive plants in three replications.

The obtained data were statistically analyzed using STATISTICA 8.0. Duncan's Multiple Range tests at a significance level ($p<0.05$) to measure specific differences between pairs of means was used.

RESULTS AND DISCUSSIONS

Figure 1 shows the minimum and maximum daily temperature inside the greenhouse through the 2023 and 2024 growing season. The average minimum daily temperature inside the greenhouse through the 2023 season was 10.16°C and 10.28°C through the 2024 season. The average maximum daily temperature inside the greenhouse through the 2023 season was 31.77°C and 37.86°C through the 2024 season.

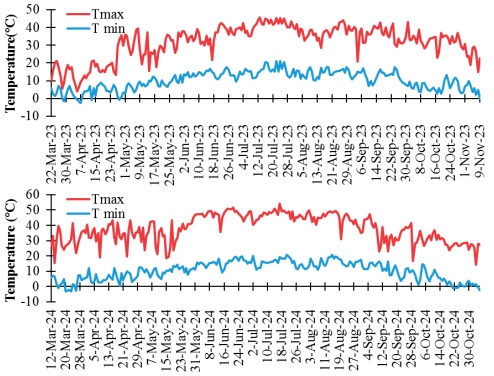


Figure 1. Minimum and maximum daily temperature throughout the 2023 and 2024 growing season

Figure 2 shows the minimum and maximum daily humidity inside the greenhouse through the 2023 and 2024 growing season. The average minimum daily humidity inside the greenhouse through the 2023 season was 37.53% and 29.08% through the 2024 season. The average maximum daily humidity inside the greenhouse through the 2023 season was 87.98% and 83.55% through the 2024 season.

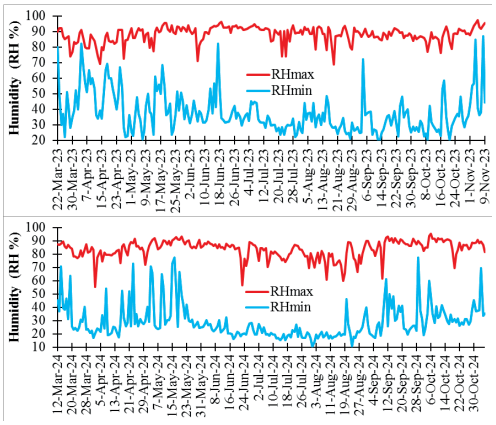


Figure 2. Minimum and maximum daily humidity throughout the 2023 and 2024 growing season

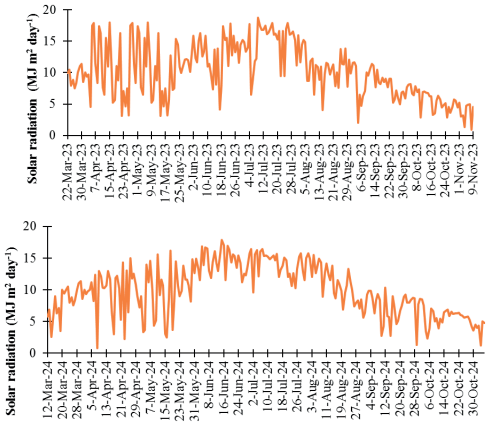


Figure 3. Daily solar radiation throughout the 2023 and 2024 growing season

Figure 3 shows the daily solar radiation inside the greenhouse through the 2023 and 2024 growing season. The average daily solar radiation inside the greenhouse through the 2023 season was 10.19 MJ m^2 day^{-1} and 9.98 MJ m^2 day^{-1} through the 2024 season.

Figure 4 shows the daily reference evapotranspiration through the 2023 and 2024 growing season. The average daily reference evapotranspiration through the 2023 growing season was 2.26 mm and 2.36 mm through the 2024 growing season.

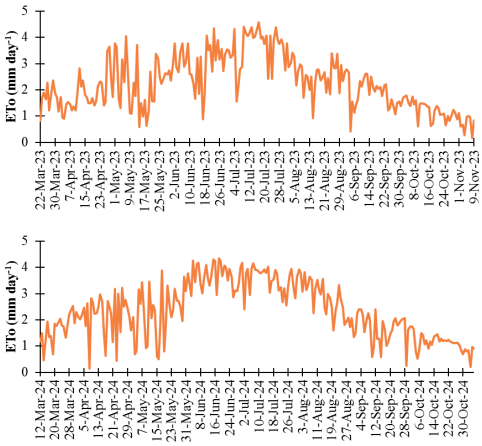


Figure 4. Daily reference evapotranspiration throughout the 2023 and 2024 growing season

The data was used to calculate daily reference evapotranspiration. Mean daily crop evapotranspiration (ETc) throughout the 2023 growing season was 1.64 mm day^{-1} and it was ranged from 0.11 mm day^{-1} (8 November 2023) to 3.66 mm day^{-1} (17 July 2023). Mean daily crop evapotranspiration throughout the 2024 growing season was 1.74 mm day^{-1} and it was ranged from 0.11 mm day^{-1} (8 April 2024) to 3.48 mm day^{-1} (18 June 2024). The ETc increased synchronized with the intensity of vegetative development of strawberries and with temperature increasing. Average ETc throughout the flowering stage (25 April 2023 - 24 May 2023) was 1.71 mm day^{-1} and 1.38 mm day^{-1} (27 March 2024 - 02 May 2024). Average ETc throughout the fruiting stage was 2.46 mm day^{-1} (25 May 2023 – 14 July 2023) and 2.08 mm day^{-1} (17 April 2024 - 17 June 2024). Lower daily average ETc throughout the 2024 season was due to earlier crop vegetative development compared to 2023 season. Total strawberry evapotranspiration for 2023 growing season was 380.99 mm and 416.34 mm for 2024 season.

The fruit size data are presented in Figure 5. The results showed that the highest mean transversal

(x) fruit size was obtained from I1F1 treatment (23.71 mm in 2023 and 22.35 mm in 2024). The lowest mean transversal (x) fruit size was obtained from I2F1 treatment (22.66 mm) in 2023 and 19.04 mm in 2024 from I2F2. There was a significant reduction (16%) in transversal (x) fruit size in the second growing year (2024) from the most unfavourable growing treatment (I2F2). While, the reduction was in the order of 5-7% from the other treatments.

The results showed that the highest mean longitudinal (y) fruit size was obtained from I1F2 treatment (20.77 mm) in 2023 and 20.75 mm in 2024 from I1F1. The lowest mean longitudinal (y) fruit size was obtained from I2F1 treatment (19.61 mm) in 2023 and 18.01 mm in 2024 from I2F2. There was a reduction (8%) in longitudinal (y) fruit size in the second growing year (2024) from the most unfavourable growing treatment (I2F2). While, from I1F1, I0F0 and I2F1 an increase was observed.

The results showed that the highest mean fruit diameter (d) was obtained from I1F2 treatment (22.23 mm) in 2023 and 21.55 mm in 2024 from I1F1. The lowest mean fruit diameter (d) was obtained from I2F1 treatment (21.14 mm) in 2023 and 18.53 mm in 2024 from I2F2. There was a significant reduction (12%) in fruit diameter (d) in the second growing year (2024) from the most unfavourable growing treatment (I2F2). While, the reduction was in the order of 2-4% from the other treatments.

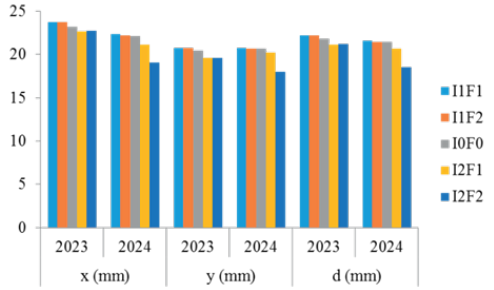


Figure 5. Fruits size by treatments throughout the 2023 and 2024 growing season

The reduction fruit sizes in the second growing year (2024) from the most unfavourable growing treatment (I2F2) probably due to depletion of plant potential and reserves. Similar results have been reported by Bibi et al. (2016) for red variety.

The fruit weight (g) and fruits number per plant data are presented in Figure 6. Fruit weight reduction was 6-9% from I0F0, I1F1 and I1F2 treatments in 2023 and 2024 growing season. The fruit weight reduction from I2F1 treatment was 16% in 2024. The fruit weight reduction from I2F2 treatment was 31% in 2024. As opposed to fruit sizes and fruit weight the fruits number per plant increased. The fruits number per plant increasing from I1F1 treatment was 84% in 2024. The fruits number per plant increasing from control I0F0 treatment was 81% in 2024. The lowest increasing was obtained from I2F1 treatment – 75% in 2024 growing season.

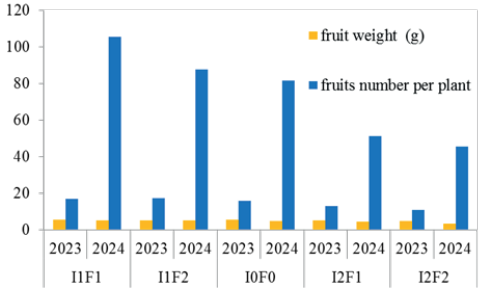


Figure 6. Fruits number per plant and fruit weight by treatments throughout the 2023 and 2024 growing season

The results (Table 1) showed that the highest mean fruit numbers was obtained from I1F2 treatment 17.27 pcs in 2023. Reduction of the fruit numbers between the highest (I1F2) and the lowest (I2F2) values was 37%. Results of Duncan's Multiple Range test show that there was significant difference between the means of I2F2 and I1F1, I1F2 and I0F0. The treatments were divided into two homogeneous groups.

Table 1. Average number of fruits per plant by treatments

Treatment	Fruits/plant (pcs)		
	2023	2024	2023-2024
I1F1	16.60a	105.33a	60.97a
I1F2	17.27a	87.67a	52.47ab
I0F0	15.60a	81.33	48.47b
I2F1	12.80ab	51.07b	31.93c
I2F2	10.93b	45.53b	28.23c
F	3.227	17.337	14.62
p	0.0188	0.000	0.000

Values with same lowercase letter for the same parameter were not statistically different.

The results (Table 1) showed that the highest mean fruit numbers was obtained from I1F1 treatment 105.33 pcs in 2024. Reduction of the fruit numbers between the highest (I1F1) and the lowest (I2F2) values was 57%. Results of Duncan's Multiple Range test show that there was significant difference between the means of control treatment I0F0 and all other treatments. The treatments were divided into three homogeneous groups.

For the two years period 2023-2024, the highest average number of fruits per plant was observed in I1F1 treatment 60.97 pcs. Reduction of the fruit numbers between the highest (I1F1) and the control treatment (I0F0) was 21%. Reduction of the fruit numbers between the highest (I1F1) and lowest (I2F2) was 54% more than the most unfavourably grown variant I2F2 (Table 1). The results from Duncan's multiple range test indicated that there was a significant difference between means of I0F0 and all other treatments excluding I1F2 at p=0.05. The treatments were divided into three homogeneous groups.

With the decrease of irrigation rate and fertigation rate, the mean fruit weight and fruits number per plant also decreases. Similar results have been reported by Kang et al. (2018), Kachwaya et al. (2015) for red variety.

CONCLUSIONS

Water and nutrient regimes are the main factors, which affect white strawberry fruit sizes, fruit weight and number of fruits per plant.

These results show that optimal irrigation and fertilization conditions lead to significantly greater fruit mass as well as number of fruits per plant. However, there is no significant difference in their mass and number between certain combinations of irrigation and fertilizer rates, such as I1F1 and I1F2, and between I1F2 and I0F0, which indicates that some combinations are interchangeable in the context of achieving greater fruit mass and number.

The significant differences between the other pairs of treatments highlight the importance of proper irrigation and fertigation management to optimize yield. As irrigation and fertigation rates decrease, the average fruit weight and number also decrease.

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FUNGAL MICROBIOTA OF EDIBLE ROSE FLOWERS

Carmen Palmira IONESCU, Ana Cornelia BUTCARU, Beatrice Michaela IACOMI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: carion2017@yahoo.com

Abstract

Flowers provide a diverse and unique habitat for microorganisms. A preliminary study on the microbiota of edible rose flowers was carried out at the USAMV of Bucharest. Rose flowers from cultivars Brother Cadfael, Crown Princess Margarett, and Falstaff were collected in October 2023 and May 2024. The composition of the fungal community associated with organic edible rose petals and stamens was analyzed. The fungal community associated with rose petals was represented by isolates of Alternaria spp. (A. alternata), Aspergillus spp. (A. niger, Aspergillus Nigri Section), Aureobasidium spp. (A. pullulans), Cladosporium spp. (C. cladosporioides), Botrytis cinerea, Epicoccum spp. (E. purpurascens), Fusarium spp. and Penicillium spp. This study provides important insights into the fungal community associated with organic edible rose flowers.

Key words*: Brother Cadfael, Crown Princess Margarett, Falstaff, fungal microbiota.*

INTRODUCTION

Plants live in close association with beneficial and pathogenic microorganisms, which together form the plant microbiota. The complex relationship between plants and their microbiota has raised fundamental questions about plant responses to these microorganisms and the main factors determining the microbiota's structure, diversity, and functionality in the soil, rhizosphere, and plant organs (Andrews & Harris, 2000).

Interest in the flower microbiome has recently intensified, but flowers colonizing microorganisms have been studied for over a century. Bacteria and fungi associated with the floral microbiome have very interesting ecological traits that influence their ability to colonize that niche, to make possible associations with flower visitors, including pollinators, and to metabolize unique nutrient sources in flowers (Vanette, 2020)

Due to their ephemerality and complex structure, flowers provide unique habitats for microorganisms, encompassing a variety of distinct niches at the microscale (Aleklett et al., 2014; Keller et al., 2021).

Before anthesis, flower buds and nectar in unopened flowers can contain detectable colonies of fungi and bacteria (Shade et al., 2013; von Arx et al., 2019). Petals of newly opened flowers also contain detectable and culturable microbial communities (Junker et al., 2011).

Flower stigmas and hypanthia of newly opened blossoms also yield culturable bacteria and fungi, with a low incidence (Pusey et al., 2009).

Different groups of microorganisms are found on or in flowers. Among the environmental ones, those observed on all flower tissues were *Micrococcus*, *Aspergillus*, and Penicillium, with low abundance and an unknown effect on flower phenotype (Morris et al., 2020; Shade et al., 2013).

Among the microorganisms found on plant surfaces, *Pseudomonas*, *Aureobasidium*, *Rhodotorula*, and *Cryptococcus* have been reported (Farré-Armengol et al., 2016).

Plant pathogens and plant endophyte are represented by isolates of bacteria (*Erwinia*, *Xanthomonas, Streptomyces*) and fungi as *Alternaria*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Monilinia*, and *Microbotryum* (An et al., 2020; Kim et al., 2019).

Flowers are likely the primary habitat for *Acinetobacter*, *Rosenbergiella*, *Metschnikowia reukaufii*, and *Metschnikowia gruessi*. These bacteria and fungi are predominantly isolated from flowers or flower-related habitats. They are numerically dominant in nectar, pollen, and pistils and contribute to the abundant growth and metabolism of nectar and floral resources

(Herrera et al., 2008; Schaeffer & Irwin, 2014; Yang et al., 2019).
The complex, multiple relationships of the plant with pollinators and other flower visitors are also to be considered. Different pollinators can promote or suppress the growth of certain microorganisms colonizing the flowers.
Animals associated microorganisms (commensal or beneficial) that are found in both flowers and animals and which use floral resources as petals, nectar, and pollen are bacteria (*Lactobacillus*) and fungi (*Kodamaea, Metschnikowia, Starmerella, Wickerhamiella*). These communities are not often isolated or frequently numerically dominant in flowers (McFrederick et al., 2012). Some can metabolize floral resources, while others may have a short life span on flowers.
Current understanding of which, when, and how microbial communities form on and within flowers, how associated microbiomes affect floral phenotypes and plant-pollinator interactions, and how these communities disperse out of flowers is reviewed by Vanette (2020). Floral traits, such as morphology and age (Morris et al., 2020), plant nutrient availability, plant chemistry, volatile and non-volatile (Boachon et al., 2019) as well as the presence of visitors, arrival order of flowers visitors (Morris et al., 2020; Vannette & Fukami, 2017) or environmental exposure (Figueroa et al., 2019) and environmental drivers as pollutants, chemicals (Schaeffer et al., 2017) can influence microbial growth on flowers.
In this context, our studies focus on exploring the cultivable microbiota of edible rose flowers, with the aim of better understanding the composition of these microbial communities.

MATERIALS AND METHODS

In October 2023 and May 2024, flowers were harvested from the three varieties of edible roses from the USAMV Bucharest orchard: Brother Cadfael, Crown Princess Margarett, and Falstaff (Figure 1).
Brother Cadfael rose variety is characterized as a vigorous bush with heights of 2.5-3m, large flowers with pink petals (Figure 2), and remontant flowering that can last until the beginning of November.
Crown Princess Margarett is a climbing rose with apricot-coloured petals (Figure 2) and a strong fruity scent. It has vigorous growth, reaching 3.5-4 m in height, single flowers or inflorescences, and remontant flowering.
Falstaff is a climbing rose, with carmine-red petals (Figure 2) and a strong scent of old roses. It has a vigorous bush, reaching 250-300 cm in height, remontant flowering, and large flowers with many petals.



Figure 1. Edible rose plantation at USAMV Bucharest

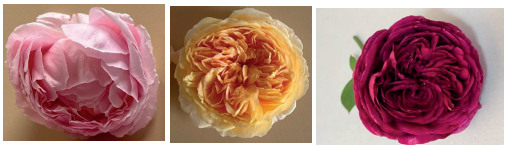


Figure 2. Edible rose varieties: Brother Cadfael, Crown Princess Margaret, and Falstaff

The detection, isolation, and identification of rose flower microbiota were done by incubating petals and stamens in a PDA culture medium (Potato Dextrose Agar, Scharlau).
Petal samples sectioned into small fragments (0.5/0.5 cm) and stamens were placed in Petri dishes on PDA culture medium (Scharlau) and incubated at 22°C.
Colonies developed were identified based on their morphology, followed by microscopic examination. Results are expressed as the incidence (%) of identified isolates (the number of isolates compared to the total number of analyzed fragments) and the incidence of bacterial or fungal colonies (%).

RESULTS AND DISCUSSIONS

In October 2023, bacteria and fungi represented the cultivable microbiota of the Brother Cadfael variety's petals. Compared to the total number of colonies, the incidence of bacteria was 65.6%. Fungi recorded an incidence of 34.4%. The incidence of detected isolates is

shown in Figure 3. Thus, the high incidence of bacterial colonies (80%) is highlighted.

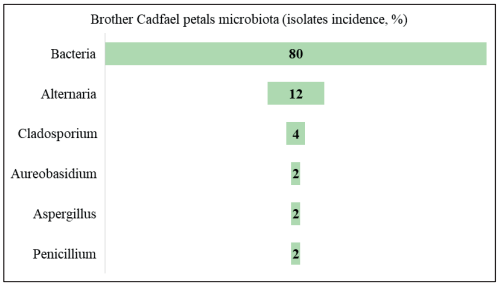


Figure 3. Petal microbiota (incidence of isolates, %), Brother Cadfael variety (October 2023)

Among the detected and identified fungal colonies, those belonging to the genus *Cladosporium* were the majority (24%), followed by those of *Alternaria* (12%). Isolates of the genera *Aspergillus* and *Penicillium* were also identified, with an incidence of 2%, and isolates of the genus *Aureobasidium*.

In samples taken in May 2024, no colonies developed from the incubated fragments were detected.

The microbiota of the colonies detected in Brother Cadfael petals is shown in Figure 4.

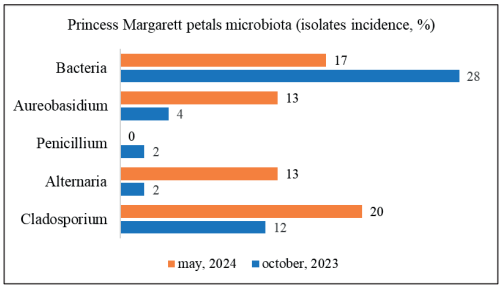


Figure 4. Brother Cadfael petals microbiota in October 2023

In October 2023, the microbiota associated with flower petals of the Crown Princess Margarett variety was represented by isolates from the genera *Cladosporium* (12% incidence), *Aureobasidium* (4% incidence), *Alternaria*, and *Penicillium* (2% incidence). Bacterial colonies were also detected with a 28% incidence (Figure 5). In May 2024, isolates from the genera *Cladosporium* (20% incidence), *Aureobasidium* (13% incidence), and *Alternaria* (13% incidence) were detected.

Some aspects of the detected isolates' morphology are shown in Figure 6.



Figure 5. Petals microbiota, Crown Princess Margaret variety (October 2023 and May 2024)

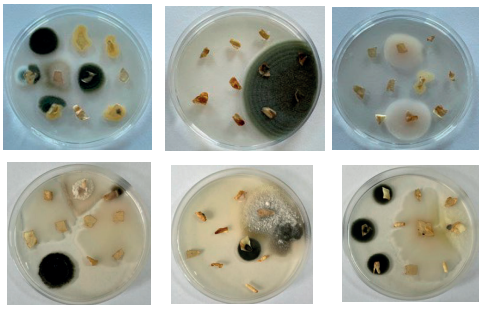


Figure 6. Crown Princess Margaret petals microbiota

In October 2023, the petals microbial community of the Falstaff variety was generally represented by bacterial colonies, with an incidence of 70% (Figure 7). In May 2024, the microbiota was represented by isolates from the genera *Cladosporium* (33% incidence), *Alternaria* (30% incidence), and *Aureobasidium* (3% incidence). Bacterial colonies were also detected (17% incidence). The morphology of some of the colonies detected in the Falstaff petals microbial community is shown in Figure 8.

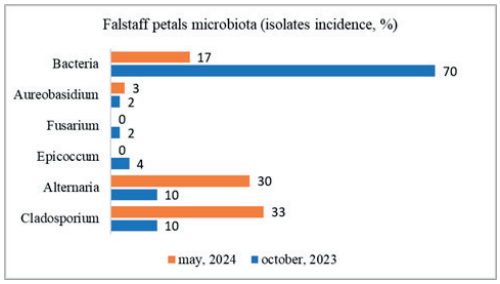


Figure 7. Petal microbiota, Falstaff variety

Aureobasidium, *Fusarium*, *Epicoccum*, *Alternaria*, and *Cladosporium* isolates were recorded among the fungi.

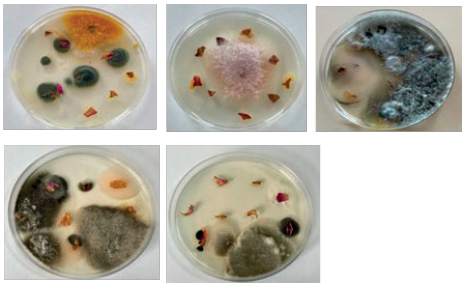


Figure 8. Falstaff petals microbiota in October 2023

The results regarding the microbiota detected and identified at the stamen level are presented in Table 1. In the samples analyzed in October 2023, no significant differences in microbial community were observed between the three tested varieties. Generally, the microbiota was represented by bacterial and fungal colonies, like in the petal variants. The presence of *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, and *Botrytis* colonies was recorded among the colonizing fungi. We report the presence of isolates of *Botrytis cinerea* at the stamen levels, the pathogen known to attack the flowers.

The microflora was less represented in the May 2024 samples. Thus, no colonies developed from the incubated fragments in the Brother Cadfael variety.

Only colonies of the *Cladosporium* genus were detected in the Crown Princess Margaret variety. In the Falstaff variety, colonies of the *Alternaria*, *Aureobasidium*, and *Cladosporium* genera and bacterial colonies have been developed.

In October 2023, the petals microbial community of the Falstaff variety was mainly represented by bacterial colonies, with an incidence of 70% (Figure 7).

Table 1. Rose stamen microbiota

Microbial community	Brother Cadfael		Crown Princess Margaret		Falstaff	
	Oct. 2023	May 2024	Oct. 2023	May 2024	Oct. 2023	May 2024
Fungi						
Alternaria						
Aspergillus						
Aureobasidium						
Cladosporium						
Botrytis						
Penicillium						
Bacteria						
Bacteria						

The morphology of the colonies detected in October 2023 is shown in Figure 9.

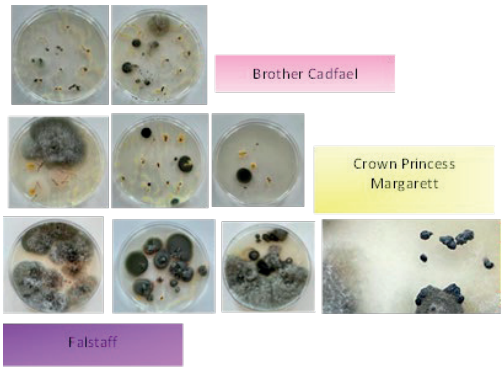


Figure 9. Rose stamen microbiota

Our research highlighted the cultivable microbiota of organic edible rose flowers at the level of petals and stamens.

The fungal microbial community associated with the petals was represented by isolates belonging to the genera *Alternaria* (*A. alternata*), *Aspergillus* (*A. niger*), *Aureobasidium* (*A. pullulans*), *Cladosporium* (*C. cladosporioides*), *Botrytis* (*B. cinerea*), *Epicoccum* (*E. purpurascens*), *Fusarium* and *Penicillium.* Bacterial colonies were the majority in October in all the tested varieties (Brother Cadfael, Crown Princess Margaret, and Falstaff).

Isolates of *Alternaria* and *Cladosporium* have been detected to have a higher incidence on petals. Also, they were detected in stamens in autumn (October 2023). *Alternaria*, *Cladosporium*, and *Botrytis* species can colonize plant tissues asymptomatically or symptomatically. They have been reported on petals, nectar, pollen, and pistils, and their presence can increase flower attractiveness. Their abundance is variable, typically low (An et al., 2020; Kim et al., 2019).

Species of *Cladosporium* (*Cladosporium cladosporioides*, *C. devikae*, *C. macadamiae*, and *C. proteacearum*) are reported as associated with flower blights (Van den Berg et al., 2008; Prasannath et al., 2021).

Isolates of *Aspergillus* (*A. niger*, *Aspergillus* Nigri species complex) and *Penicillium* have been recorded with low incidence on petals. Their presence on stamens has also been

highlighted (Morris et al., 2020; Shade et al., 2013).

Among fungal isolates, those belonging to the genera *Aureobasidium and Epicoccum* may be interested in understanding their role in the flower microbiota. These genera are known for their species, which have potential in the biological control of plant diseases.

Isolates of *Aureobasidium* are known to be present at the petals levels, impacting metabolizing plant compounds and producing volatiles or other metabolic by-products (Farré-Armengol et al., 2016). Species of the genus *Aureobasidium* produce different compounds (pullulan, β-glucan, malic and polymalic acids, melanin, lipids) used in agriculture. They are also known for their effect on plant growth and their protection. Isolates of *Aureobasidium* adhere to plant cells, producing extracellular polysaccharides, forming biofilm (Rensink et al., 2024). On the market, Blossom Protect and Botector are bioproducts formulated based on *A. pullulans* strains DSM 14940 and DSM 1494 (Andermatt Biocontrol Romania) highly effective for the prevention of fire blight in pome fruits through colonization of blossoms and nectarines and natural competition for space and nutrients or grey mould infections on grapes and berries.

Epicoccum species are ascomycetes known for their endophytic potential, which promotes plant growth and protects plants from other pathogens (Favaro et al., 2012; de Cal et al., 2009).

It can be assumed that the presence of *Aureobasidium* and *Epicoccum* isolates in the flower microbial community may be linked to controlling natural pathogens. Further studies are needed to examine whether this community diversity, abundance, and/or species composition could enhance protection against floral pathogens and which biotic or abiotic conditions affect microbiome-mediated pathogen protection. Research is ongoing to characterize *Aureobasidium* and *Epicoccum* isolates in terms of antagonistic potential.

CONCLUSIONS

The research highlighted the cultivable microbiota associated with the flowers of organic edible roses (petals and stamens) in autumn (October) and spring (May). To our knowledge, this is the first report of a flower's microbial community.

Alternaria, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Botrytis*, *Epicoccum*, *Fusarium*, and *Penicillium* isolates represented the fungal microbial community associated with edible rose petals. A high incidence of bacterial colonies was recorded in all tested varieties (Brother Cadfael, Crown Princess Margarett, and Falstaff).

Alternaria, *Aspergillus*, *Aureobasidium*, *Cladosporium*, and *Botrytis* isolates represented the stamen microbiota. Our research highlights the presence of isolates of *Botrytis cinerea* at the stamen level.

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GENETIC DIVERSITY AND RELATIONSHIP ASSESSMENT OF ROMANIAN BLUEBERRY CULTIVARS USING SRAP MARKERS

Mihaela IORDĂCHESCU, Maria OJOG, Anca-Amalia UDRIȘTE, Liliana BĂDULESCU

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: liliana.badulescu@qlab.usamv.ro

Abstract

Sequence-related amplified polymorphism (SRAP) molecular markers have proven to be an effective tool for assessing genetic diversity and cultivar identification in various plant species. Present study aimed to genotype nine Romanian blueberry (Vaccinium corymbosum) cultivars – 'Lax', 'Prod', 'Vital', 'Azur', 'Simultan', 'Delicia', 'Pastel', 'Safir', and 'Augusta' - using SRAP markers to evaluate their genetic variability and relationships. A set of polymorphic SRAP primers was selected to amplify DNA fragments, and the banding patterns were analysed to assess genetic similarity. Cluster analysis grouped the cultivars based on their genetic similarities, providing valuable insights into their genetic background and potential breeding applications. The study reveals the effectiveness of SRAP markers for the differentiation of Romanian blueberry cultivars, providing a dependable approach for cultivar identification, genetic conservation, and breeding initiatives. This research could be a valuable support for improved cultivars development.

Key words*: SRAP markers, genetic diversity, Vaccinium corymbosum, cultivar identification, molecular genotyping.*

INTRODUCTION

Blueberry (*Vaccinium corymbosum* L.) is known for its high nutritional value, antioxidant-rich fruit, and increasing consumer demand (Duan et al., 2022; Prior et al., 1998). Recently, blueberry cultivation has expanded significantly in Romania, due to favourable climatic conditions and growing market demand for fresh fruit consumption (Asănică et al., 2017). Recently, Hera (2024) published a study detailing the origin of the Romanian blueberry cultivars. However, the genetic background of many Romanian blueberry cultivars remains poorly characterized, which limits the effectiveness of breeding programs and germplasm conservation strategies.

Several molecular marker systems have been employed to study genetic variation within the genus *Vaccinium*, including Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Simple Sequence Repeats (SSR) (Iordăchescu & Mihai, 2022). SRAP marker system is based on the amplification of open reading frames using primer pairs that contain the CCGG sequence in the forward primer and AATT sequence in the reverse primer, followed by 3 selective nucleotides at their 3' end (G. Li & Quiros, 2001). This simple system has been used for a variety of purposes, including mapping and gene tagging (G. Li & Quiros, 2001), assessment of genetic stability and somaclonal variation (Clapa et al., 2019; Ismail et al., 2022), phylogenetic studies (Ho et al., 2024), population structure analysis (Wang, 2020; Zhou et al., 2021), cultivar identification (Handa et al., 2015; Shamustakimova et al., 2021; Sun et al., 2016) and genetic diversity analysis (Y. Li et al., 2009; Szabo et al., 2021; Zagorcheva et al., 2024).

The use of SRAP markers in *Vaccinium* research is limited so far. Clapa et al. (2019) used RAPD and SRAP markers to check the genetic stability of micropropagated blackberry and blueberry (*V. corymbosum*) plants. In another study, SRAP markers and chloroplast *mat*K gene were used to determine the relationships and taxonomic status of intraspecific *Vaccinium uliginosum* L. germplasm resources located in the Changbai Mountain (Chen et al., 2022).

Present study we used SRAP markers to assess the relationships and genetic diversity among the nine Romanian blueberry cultivars. The goal of the study is to obtain data about the usefulness of SRAP markers in differentiating the cultivars and identifying genotypes that should be prioritized for conservation, as well as to

provide breeders with valuable information to be used in creating new, improved cultivars.

MATERIALS AND METHODS

Materials

The blueberry genotypes analysed in this study are the Romanian cultivars 'Lax', 'Prod', 'Vital', 'Azur', 'Simultan', 'Delicia', 'Pastel' and 'Augusta'.

Genomic DNA extraction

Genomic DNA was extracted from young leaves with the Innu PREP Plant DNA I KIT IPC 16 (Analytik Jena GmbH+Co, Jena, Germany). For the external lysis of the tissue approximately 100 mg fresh tissue was transferred to 1.5 ml tubes containing 600 µl lysis solution SLS and 20 µl proteinase K, and the tissue was disrupted with a micropestle. The samples were then incubated at 65°C for 1 hour, centrifuged at 14000 rpm for 5 min at room temperature to separate the lysed tissue, and supernatant transferred to prefilters fitted into collection tubes. The samples were centrifuges again for 2 min at 10000 x g, and 2 µl of RNase A (10 mg/ml) were added to the samples in the collection tubes, with a further 5 min incubation at room temperature. After external lysis, samples the transferred into the kit plaque and processed further in the InnuPure C16 instrument (Analitik Jena), using the Ext_Lysis_200_C16_04 program. DNA quality and quantity were evaluated with NanoDrop 1000 spectrophotometer (BioRad, California, USA). Genomic DNA integrity was checked electrophoresis on 1% agarose gel in 1xTAE buffer, and visualised with the Pharox FX system (BioRad, California, USA).

Polymerase Chain Reaction

Five primer pairs were used as SRAP markers: ME1-EM1, ME2-EM2, ME3-EM3, ME4-EM4, and ME5-EM5 (Table 1).

Annealing temperature optimization was performed for each pair of SRAP primers and the results compared to those obtained by standard amplification (G. Li & Quiros, 2001) modified to fit with the Platinum II Hot Start kit (Table 3). Once it was decided the optimum annealing temperature for each pair of SRAP primers, the reactions were done in triplicate and only bands present in all reactions for each SRAP marker were scored.

Table 1. SRAP Markers

SRAP Marker	Primers	DNA Sequence (5'- 3')
ME1-EM1	ME1	TAGGTCCAAACCGGAAG
	EM1	GACTGCGTACGAATTAAT
ME2-EM2	ME2	TGAGTCCAAACCGGACA
	EM2	GACTGCGTACGAATTACA
ME3-EM3	ME3	TGAGTCCAAACCGGACT
	EM3	GACTGCGTAGGAATTACT
ME4-EM4	ME4	TGAGTCCAAACCGGAGA
	EM4	GACTGCGTACGAATTACA
ME5-EM5	ME5	TAGGTCCAAACCGGATC
	EM5	GACTGCGTACGAATTATC

PCR reactions were performed using the Platinum II Hot Start kit (Invitrogen) according to the manufacturer instructions. PCR setup was done according to Table 2.

Table 2. PCR reaction setup

Component	Volume	Final Concentration
Platinum™ II HotStart PCR Master Mix (2x)	5.0 µl	1x
10 µM forward primer	0.2 µl	0.2 µM
10 µM reverse primer	0.2 µl	0.2 µM
10 ng/µl template DNA	2.0 µl	2 ng/µl
Nuclease-free water	2.6 µl	-
Total	10.0 µl	-

Table 3. PCR cycling conditions

	Temperature	Time	Cycles
Standard SRAP amplification	94°C	2 min	1
	94°C	15 sec	5
	35°C	1 min	
	68°C	1 min	
	94°C	15 sec	30
	50°C	1 min	
	68°C	1 min	
	68°C	10 min	1
	4°C	HOLD	
Annealing temperature optimization	94°C	2 min	1
	94°C	15 sec	30
	47-57°C	1 min	
	68°C	1 min	
	68°C	1 min	1
	68°C	10 min	1
	4°C	HOLD	

Data Analysis

Polymorphism levels of SRAP markers were assayed based on the percentage of polymorphic loci, cumulative Shannon's information index, and polymorphism information content (PIC). Percentage of polymorphic loci was calculated using the formula:

$$Percentage\ of\ Polymorphic\ loci = \frac{Nr.\ of\ polymorphic\ loci}{Total\ nr.\ of\ loci} \times 100$$

Shannon information index, that quantifies the diversity in the presence/absence of a DNA fragment across genotypes was calculated for

each locus using the formula $H = -(p \cdot log_2(p) + (1 - p) \cdot log_2(1 - p)$, where p represents the frequency of band presence and 1-p represents the frequency of band absence at a certain locus.

Polymorphism information content (PIC) was calculated using the formula $PIC = 2pq = 2p(1-p)$ (Serrote et al., 2020), where p represents the frequency of band presence and $q=1-p$ represents the frequency of band absence at a certain locus. Amplified DNA was scored as present (1) or absent (0) in a *.csv file as a binary matrix. Data was then analysed with BIO-R software, Biodiversity Analysis with R for Windows, version 3.2, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and Microsoft® Excel® for Microsoft 365 MSO (Version 2406 Build 16.0.17726.20078) 64-bit.

RESULTS AND DISCUSSIONS

Annealing temperature optimization

Following the comparison of the results of the standard SRAP amplification and annealing temperature optimization reactions it was decided that for the ME1-EM1 and ME2-EM2 it will be used a marker specific annealing temperature (46°C and 48°C) and for the ME3-EM3, ME4-EM4, and ME5-EM5 markers will be used the standard SRAP amplification parameters.

SRAP markers assessment

The SRAP markers amplified a total of 59 loci, out of which 37 were polymorphic (Table 4).

Table 4. SRAP markers efficiency

SRAP Marker	Total loci	Polymorphic loci	Percentage of polymorphism	Cumulative Shannon Index	Mean PIC across all loci	Mean PIC across polymorphic loci only
ME1-EM1	7	4	57.14	2.07	0.20	0.35
ME2-EM2	10	8	80.00	4.43	0.30	0.37
ME3-EM3	17	10	58.82	5.39	0.21	0.36
ME4-EM4	11	6	54.55	3.73	0.24	0.44
ME5-EM5	14	9	64.29	4.60	0.22	0.33

The number of total loci ranged from 7 (ME1-EM1) to 17 (ME3-EM3), whereas the number of polymorphic loci varied from 4 (ME1-EM1) to 10 (ME3-EM3). The polymorphism was above 50% for all markers, with the highest percentage of polymorphism (80%) detected for the marker ME2-EM2. ME1-EM1 marker has the lowest percentage of polymorphism (57.14%) and the lowest value of the cumulative Shannon index (2.07), which indicates that it has a limited power to detect polymorphism. On the other hand, ME3-EM3 marker displayed the highest degree of polymorphism, the highest cumulative Shannon index (5.39), and it amplified DNA on the highest number of total loci.

PIC values for dominant markers, such as SRAPs, cannot be higher than 0.5 when using the $PIC = 2pq = 2p(1-p)$ formula, when the allele frequencies are equal (p=q=0.5). In this case, Serrote et al. (2020) proposed for dominant markers informativeness the following ranges: low (0.00-0.10), medium (0.10-0.25), high (0.30-0.40) and very high (0.40-0.50). For the markers studied here, mean PIC values across all loci ranged between 0.20 (ME1-EM1) and 0.30 (ME2-EM2), indicating a medium to high informativeness for all markers. However, if only polymorphic loci are taken into consideration, the PIC values increase, ranging from 0.33 (ME5-EM5) to 0.44 (ME4-EM4), high to very high informativeness.

When looking at the total polymorphism, Shannon diversity index, and PIC values, ME2-EM2 appeared to be the best informative marker, with the polymorphism (80.00%), the highest mean PIC across all loci (0.30), and a strong cumulative Shannon Index (4.43), making it ideal for broad diversity analysis and germplasm differentiation. The ME3-EM3 marker, has slightly lower polymorphism (58.82%) and moderate PIC values, but the highest cumulative Shannon Index (5.39), and amplified the greatest number of loci (17). Therefore, this marker can be used successfully for overall genetic diversity assessment. The ME4-EM4 marker produced fewer polymorphic loci (6) and had a moderate Shannon Index (3.73), but showed the highest PIC value across polymorphic loci (0.44). This indicates that this marker can be used for studies requiring precise identification of cultivars.

Genetic diversity analysis

One of the ways to estimate the genetic difference between two genotypes is to calculate Rogers' genetic distances (Mohammadi & Prasanna, 2003). Analysis of the binary data with the Bio-R software generated the Rogers' distances between the blueberry genotypes (Table 5).

Table 5. Rogers distances between blueberry genotypes

NAME	gLax	gProd	gVital	gAzur	gSimultan	gDelicia	gPastel	gSafir	gAugusta
1. gLax	0	0.69749	0.69749	0.69749	0.36761	0.73521	0.56949	0.67783	0.56949
2. gProd	0.69749	0	0.51988	0.69749	0.67783	0.69749	0.73521	0.54525	0.56949
3. gVital	0.69749	0.51988	0	0.61512	0.67783	0.6576	0.61512	0.63671	0.61512
4. gAzur	0.69749	0.69749	0.61512	0	0.63671	0.6576	0.6576	0.59275	0.61512
5. gSimultan	0.36761	0.67783	0.67783	0.63671	0	0.63671	0.59275	0.61512	0.59275
6. gDelicia	0.73521	0.69749	0.6576	0.6576	0.63671	0	0.80539	0.59275	0.61512
7. gPastel	0.56949	0.73521	0.61512	0.6576	0.59275	0.80539	0	0.78843	0.6576
8. gSafir	0.67783	0.54525	0.63671	0.59275	0.61512	0.59275	0.78843	0	0.63671
9. gAugusta	0.56949	0.56949	0.61512	0.61512	0.59275	0.61512	0.6576	0.63671	0

Colour scale ranges from blue (low distance, genetically similar genotypes) to red (high distance, genetically distant genotypes).

Rogers distance values among the cultivars ranged from 0.3676 (between 'Simultan' and 'Lax') to 0.8054 (between 'Pastel' and 'Delicia'), identifying closely related as well as distantly related genotypes.

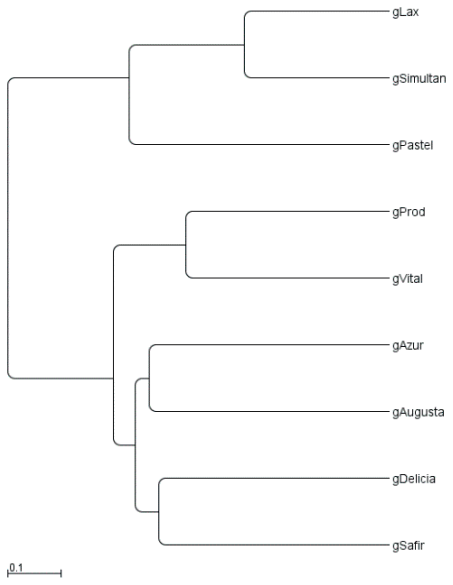


Figure 1. Hierarchical clustering dendrogram based on Rogers' distances, generated by the Bio-R software

A dendrogram based on the Rogers' distances is presented in Figure 1. The genotypes are grouped into two main clusters, the first cluster with the 'Lax', 'Simultan', and 'Pastel' cultivars, and the second cluster with the rest of the cultivars. The low genetic distance between 'Simultan' and 'Lax' indicates that the two genotypes are closely related. Besides this pair, in the second cluster, 'Prod' is grouped with 'Vital', 'Azur' with 'Augusta', and 'Delicia' with 'Safir', indicating close relationships between the pairs. Indeed, 'Lax', 'Simultan', and 'Pastel' were obtained by open pollination of 'Spartan' cultivar, and 'Azur' and 'Augusta' from a cross between 'Berkeley' and 'Bluecrop' cultivars (Hera, 2024).

2D principal component analysis (PCA) results are presented in Figure 2, illustrating the relationships between the blueberry cultivars as well. CP1 component explains 27.6% of the genetic variation and separates the 2 clusters from the dendrogram – on the right side the first cluster, and on the left side the second cluster. CP2 component explains 19.4% of the genetic variation, further grouping the blueberry cultivars.

'Pastel' cultivar, is the most genetically dissimilar, when compared to the other cultivars.

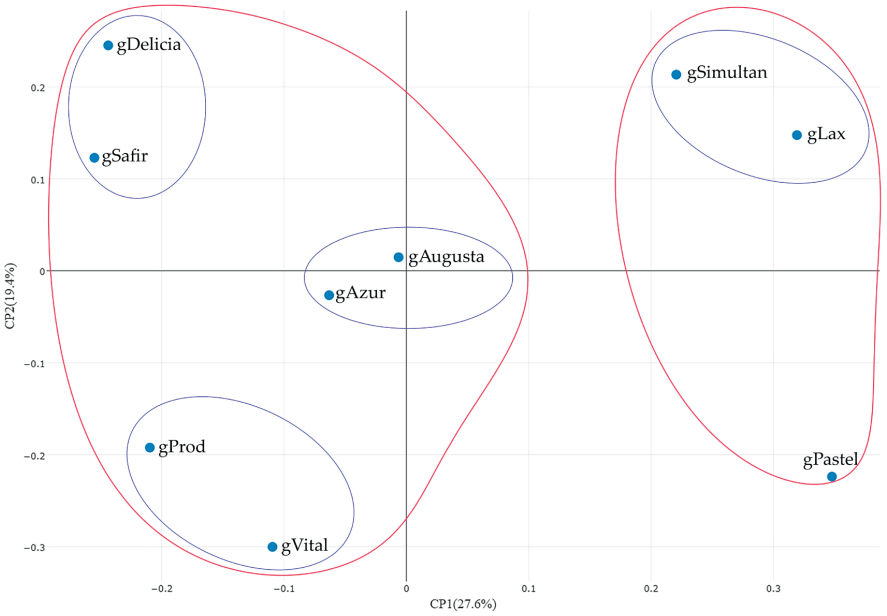


Figure 2. 2D PCA plot based on Rogers' distances, generated by the Bio-R software. Pairs of genotypes from the dendrogram are circled with blue, and the 2 clusters are circles with red.

This cultivar has some alleles that are present in few other genotypes, and should be conserved to preserve the genetic diversity, since such diversity is essential for the development of new improved cultivars that need to cope with the changing environment and answer the customers everchanging demands (Salgotra & Chauhan, 2023).

CONCLUSIONS

The five SRAP markers used were effective in differentiating the nine Romanian blueberry cultivars and revealed high levels of polymorphism (54-80%), and high genetic variation among cultivars. Among them, ME2-EM2 and ME4-EM4 markers are the most suitable for cultivar identification, due to their high degree of polymorphism and high PIC values. In terms of breeding, the same markers should be used to select genitors for crosses to introduce new traits. As the marker ME3-EM3 has the highest cumulative Shannon index, hence capturing the most diversity, it should be used for genetic conservation studies by monitoring diversity over time, and safeguarding against genetic loss.

The nine Romanian cultivars were grouped into 2 main clusters, and further into pairs, except for the cultivar 'Pastel', that has the most genetically diverse genotype, making it a good candidate for breeding programs as a genitor, and a priority for conservation.

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STUDY ON THE POSSIBILITY OF PROCESSING WHITE STRAWBERRY (*FRAGARIA* × *ANANASSA* 'SNOW WHITE') FRUITS INTO PRODUCTS FOR MASS CONSUMERS AND AS COMPONENT COMPOSITIONS IN THE BAKERY AND CONFECTIONERY INDUSTRY

Petya IVANOVA[1], Elena GRANCHAROVA[2], Blagoj ELENOV[2], Velichka YANAKIEVA[3]

[1]Institute of Food Preservation and Quality, 154 Vasil Aprilov Blvd, 4003, Plovdiv, Bulgaria
[2]Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", 7 Shosse Bankya Str., 1331 Sofia, Bulgaria
[3]University of Food Technologies, 26 Maritsa Blvd, 4002, Plovdiv, Bulgaria

Corresponding author email: petjofi@gbg.bg

Abstract

This study examines the possibility of developing products from white strawberries, considering their short harvesting and storage period. Two product assortments - white strawberry jam and white strawberry with quince (Chaenomeles) jam - were developed and analysed at the Institute of Food Preservation and Quality in Plovdiv. The fruits were cultivated and provided by the experimental field in Chelopechene, part of the Institute of Soil Science, Agrotechnologies, and Plant Protection "Nikola Poushkarov" in Sofia. The jam production technology was modified based on the component composition of the product containing white strawberries and quince. The obtained products were analysed at the Laboratory of the Institute of Food Preservation and Quality in Plovdiv and the Department of Microbiology at the University of Food Technologies in Plovdiv. The analysis included physicochemical, biochemical, and microbiological indicators in compliance with current food safety and quality legislation. The final products are intended for mass consumers and as potential components in the confectionery and bakery industries.

Key words*: white strawberry Fragaria × ananassa* 'Snow White'*, Chaenomeles, technology, products, biochemical, microbiological indicators.*

INTRODUCTION

The white strawberry (*Fragaria* × *ananassa*) is biologically identical to the common strawberry and belongs to the same species. The 'Snow White' variety was developed in 2010 by crossbreeding *Fragaria* × *ananassa* 'Weisse Ananas' and *Fragaria chiloensis* f. *chiloensis*.

The fruits of this remontant white strawberry variety are smaller, rounded, and conical, measuring between 15 and 33 mm. Their color ranges from white to creamy, with pale pink shades and red seeds. At the beginning of ripening, the fruits are green, turning white when fully ripe, while the seeds become deep red. The flesh is soft, juicier than common strawberries, colored from white to pale pink, with a sweet-sour taste and a strong aroma. The absence of bioflavonoids (biologically active plant polyphenolic compounds known as flavonoids) results in the lack of the characteristic red hue in ripe fruits. The deficiency of one of the main ripening proteins in strawberries, *Fra a1* (commonly known as *Fragaria* allergen A1), allows consumers with strawberry allergies to consume white strawberries without experiencing side effects or allergic reactions.

The quality and nutritional value of the fruit depend on the applied agrotechnical cultivation technologies. Strawberries of the species *Fragaria* × *ananassa* are an important dietary source of vitamins, minerals, fiber, and other bioactive compounds that have beneficial effects on human health. Specific compounds, such as phenols present in these fruits, exhibit various biological activities, including antioxidant, neuroprotective, anticancer, anti-inflammatory, and antimicrobial properties (Moyer, 2002; Cirico et al., 2006; Galli et al., 2006; Markovic et al., 2000; Ramirez-Tortosa et al., 2001; Karakaya et al., 2004).

White strawberries contain carbohydrates (9.16%), proteins (0.66%), fats (0.26%), citric acid (1.08%), ascorbic acid (1354 mg/kg), total carotenoids (6.6 μg/100 g), pectins (0.96%),

among other compounds. Their energy value is 173.64 kJ/42 kcal.

Chaenomeles sp., a perennial shrub from the *Rosaceae* family, is widely recognized as an ornamental plant. However, its fruits possess high biological value due to their biochemical composition: phenolic compounds (over 500 mg/100 g), leucoanthocyanins and anthocyanins (over 700 mg/100 g), vitamin C (124-182 mg/100 g), potassium (85.5 mg%), calcium (22.7 mg%), magnesium (12.0 mg%), and phosphorus (27.4 mg%). Researchers such as Chakhovsky et al. (1986), Petrova (1987), Mezhensky (1989), Hellín et al. (2003), Krasnova et al. (2007), and Mihova et al. (2012) have focused their studies on the valuable properties of this fruit.

The fruits of *Chaenomeles* sp. have a relatively high yield and are a valuable source of nutrients essential for human health, including organic acids (up to 5.5%), pectin (1.4 g/ 100 g), dietary fiber, and aromatic compounds (Kumpan & Sukhotskaya, 2010). They are also considered a non-traditional ingredient in food product development in Bulgaria (Mondeska, 2005; Hellín et al., 2003).

The aim of this study is to explore the possibility of processing white strawberry fruits into an innovative product with excellent taste qualities, intended for mass consumers and as a component in the bakery and confectionery industries, considering the fruit's short storage period.

MATERIALS AND METHODS

Raw Materials

White Strawberry – The fruits were grown in an experimental field of the Institute of Soil Science, Agrotechnologies, and Plant Protection "Nikola Poushkarov" in Chelopechene, Sofia. They were cultivated in an unheated greenhouse under 75% drip irrigation and 100% fertigation ($N_{8.09}P_{12.76}K_{15.62}$).

Chaenomeles sp. Fruits – The fruits were harvested from the demonstration plantation of the Institute of Mountain Livestock Breeding and Agriculture (IMLBA) in Troyan.

Experimental Design

Production Process of White Strawberry Jam - the jam was prepared using the following technological stages: reception of the fruits; sorting and washing; removal of stems from the white strawberries; crushing; addition of sugar; boiling to 60-62% dry matter (refractometric method); addition of pectin; •pH adjustment with citric acid; boiling to 65% dry matter; hot filling at t = 80-85°C; sealing; pasteurization at t = 96°C for 10-15 minutes; cooling and storage at t = 20-25°C.

Production Process of White Strawberry and *Chaenomeles* Jam - the jam was produced using the following technological steps: reception of the fruits; sorting and washing; removal of seeds from *Chaenomeles* fruits and stems from white strawberries; crushing; addition of sugar; boiling to 65% dry matter; hot filling at t = 80-85°C; sealing; pasteurization at t = 96°C for 10-15 minutes; cooling and storage at t = 20-25°C

Methods

Dry Matter (Refractometric), % - BDS EN 12143-2000;

Dry Matter (Gravimetric), % - BDS 7133:1981;

Active Acidity (pH) - BDS 11688-1993;

Color Measurement - Determined instrumentally using a colorimeter (PCE-CSM 5 portable colorimeter - measurement geometry 8°/d, Ø 8 mm, light source D65). The parameters were recorded using the CIE Lab system, measuring three color coordinates:

- L - Brightness (L=0 - black, L=100 - white);
- a - Positive values indicate the red component, negative values indicate the green component;
- b - Positive values indicate the yellow component, negative values indicate the blue component (ASTM D2244-16);

Carbohydrates, % - BDS 7169-1989;

Fats, % - BDS 6997-1984;

Proteins, % - BDS 14431-1978;

Total Titratable Acidity, % - BDS EN ISO 12147-2000;

Ascorbic Acid, mg% - BDS 11812-1991;

Energy Value, kJ - According to EC Regulation 1669/2011, Chapter IV, Section III, Article 31, Annex IV, and Bulgarian Ordinance No. 23/2001 of the Ministry of Health;

Organoleptic Evaluation - ISO 13299-2016;

Mesophilic Aerobic and Facultative Anaerobic Microorganisms, cfu/g - BDS 6916-1987;

Mesophilic Anaerobic Microorganisms, absence in 1 g - BDS 6916-1987;

Molds and Yeasts, cfu/g - BDS 6916-1987.

The presented results are arithmetic mean values from at least three parallel determinations, with variation coefficients of less than 5%. The statistical processing of the data was performed using variance analysis in Microsoft Excel.

RESULTS AND DISCUSSIONS

The data from the conducted tests on the raw materials and the final products - white strawberry jam and white strawberry and *Chaenomeles* jam - are presented in Figures 1, 2, 3, 4, and Table 1.

The physicochemical analysis of the two developed jams indicates that the soluble and insoluble dry matter content shows no statistically significant difference ($p > 0.05$), with values exceeding 65% and 75%, respectively (Figure 1). These values meet the minimum permissible dry matter content for this type of product (not less than 60%).

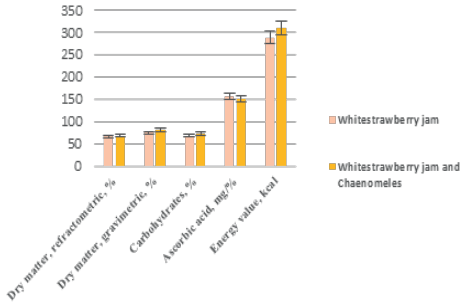


Figure 1. Physicochemical indicators of white strawberry products and white strawberry and *Chaenomeles*

Through the technological step of acidity correction - using citric acid for the first variant (white strawberry jam) and the natural acidity of *Chaenomeles* for the second variant (white strawberry and *Chaenomeles* jam) - the final products achieve an active acidity level above 4.15 (Figure 2).

With the applied sterilization regime at this acidity level, a microbiologically safe product is obtained, as confirmed by the results of the microbiological tests.

Water activity and the microenvironment within food influence bacterial growth and enzymatic processes. Most bacteria thrive within a water activity range of 0.90-1.00, yeasts at 0.94, and molds at 0.80-0.85. In the developed jams, the water activity value is above 0.71, which suggests an absence of bacterial, mold, and yeast growth (Figure 2).

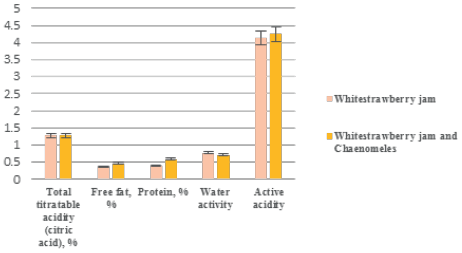


Figure 2. Biochemical indicators of white strawberry products and white strawberry and *Chaenomeles*

When analyzing the color characteristics of the products, only the red color tone parameter showed statistically indistinguishable values above 10 ($p > 0.05$).

Due to the composition of the products and the specific color of the fruits, the brightness parameter is higher in the white strawberry and *Chaenomeles* jam compared to the white strawberry jam. A comparative analysis shows that in terms of yellow color tone and qualitative color parameters - color saturation and hue - the values in the white strawberry jam are higher compared to the white strawberry and *Chaenomeles* jam (Figure 3).

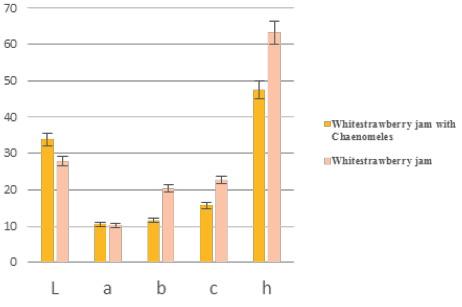


Figure 3. Color indicators of white strawberry products and white strawberry and *Chaenomeles*

The results of the biochemical indicators for the two developed products showed that the values for total carbohydrates (over 68%), acidity (1.28%), ascorbic acid (over 151 mg%), and energy value (over 289 kcal) were statistically indistinguishable from each other ($p > 0.05$) (Figures 1 and 2). Due to the specific chemical composition of *Chaenomeles*, slightly higher values were found in the white strawberry and *Chaenomeles* jam compared to the white strawberry jam for the fat and total protein indicators, with values of 0.47% and 0.59%, respectively (Figure 2).

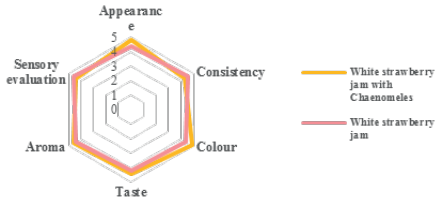


Figure 4. Sensory indicators of white strawberry products and white strawberry and *Chaenomeles*

Figure 4 presents the average ratings from the sensory tests conducted on five indicators – appearance, consistency, color, taste, and aroma of the two developed products, provided by trained tasters. For both assortments of white strawberry jam and white strawberry and *Chaenomeles* jam, the average ratings for the above-mentioned indicators were found to be above 4, statistically different ($p<0.05$), with the only exception being the consistency indicator, where the white strawberry jam received a higher rating. The maximum average ratings were for appearance and color, with the white strawberry and *Chaenomeles* jam scoring 4.82 and 4.86, respectively. The specific taste and aroma of *Chaenomeles* fruit, when used as a component in the white strawberry jam, enrich the sensory perception of the innovative product. The sweet-sour aftertaste of the white strawberry and *Chaenomeles* jam was preferred by the tasters compared to the white strawberry jam.

The data from the microbiological indicators of the developed products, analyzed in the Department of Microbiology at UHT, are presented in Table 1.

The table shows that no mesophilic anaerobic microorganisms were detected, and mesophilic aerobic and facultative anaerobic microorganisms, molds, and yeasts were below the established limits, respectively 100 cfu/g and < 10 cfu/g (Methodological guidelines for national microbiological criteria for food products not covered by EU Regulation 2073/2005 and EU Regulation 1441/2007). The applied pasteurization regime for the developed products guarantees their safety and quality.

Table 1. Microbiological indicators of white strawberry jam and white strawberry and *Chaenomeles* jam

Indicators	White Strawberry Jam	White Strawberry and *Chaenomeles* Jam
Mesophilic aerobic and facultative anaerobic microorganisms, cfu/g	<10	<10
Mesophilic anaerobic microorganisms, absence in 1 g	Not detected	Not detected
Molds and yeasts, cfu/g	<10	<10

CONCLUSIONS

Products made from white strawberry (*Fragaria* × *ananassa* 'Snow White') and a combination of white strawberry (*Fragaria* × *ananassa* 'Snow White') and quince (*Chaenomeles* sp.) have been developed, aiming to demonstrate the technological potential of using white strawberry fruits in products for mass consumption and as ingredient compositions in the bakery and confectionery industries.

It was found that the innovative products have high sensory scores, meeting the requirements of the Regulation on the specifications for fruit jams, jellies, marmalades, jelly-marmalades, and sweetened chestnut puree, adopted by Decision No. 45 from 21.02.2003, published in the Official Gazette No. 19 on 28 February 2003. These products are safe, of good quality, and suitable for consumption and inclusion in other products.

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SOME ASPECTS REGARDING FIG (*FICUS CARICA* L.) PATHOLOGY IN ROMANIA

Emanuela Diana LORENTZ[1], Florin STĂNICĂ[1], Beatrice Michaela IACOMI[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: dianalorentz@yahoo.com

Abstract

The milder winters and longer-than-normal warm periods of recent years in Romania have increased the popularity of the fig trees (Ficus carica L.) among fruit growers. Fig trees have proven to be quite resistant to diseases and pests in our continental temperate conditions. However, in recent years, several foliar diseases have been observed. Our research focused on detecting and identifying main fig diseases on the existing germplasm collection of the Faculty of Horticulture, USAMV of Bucharest. Our results highlight the presence of Fig Mosaic Disease (FMD) and brown spot (Passalora trichophila) on leaves. Black mold (Aspergillus niger) and fruit mold (Cladosporium sp.) have been identified on fig fruits. Also, the pathogen Fusarium oxysporum has been detected on fig cuttings with symptoms of wilting and basal rot. To our knowledge, this is the first report in Romania of FMD, Passalora trichophila, and Fusarium oxysporum. Further studies are needed to identify the complex of different viruses responsible for FMD in Romania.

Key words*: fig tree, Fig Mosaic Disease, Fusarium oxysporum, Passalora trichophila.*

INTRODUCTION

The fig tree (*Ficus carica* L.) is one of the first cultivated fruit trees (Vidaud et al., 1997; Aljane, 2004), being mentioned in myths and legends (Sinha, 2003), as well as in sacred texts, the Bible, Talmud and Quran (Rahmani and Aldebasi, 2017; Tomescu, 2014).

The discovery of fossilized figs, dated to 9400 - 9200 BC, suggests that the fig was cultivated at the same time as rice (Asia), but before the cultivation of wheat, barley and legumes (Kislev et al., 2006).

The fig is mentioned in the works of Herodotus, Homer, Aristophanes and Plato, while interesting information about pollination, reproduction and the benefits of fruits on human health can be found in the writing "Research on Plants" by Theophrat.

Historical sources mention that in China, the fig was cultivated, along with lemons and almonds, since 2660 BC (Tomescu, 2014). In Europe, the fig culture has been known since the 8th century.

In our country, the fig tree has been brought since Antiquity. Although the origin of the fig tree on Romanian territory is still debated, its appearance being attributed to the Greeks, Romans, Turks and Armenians, the introduction of figs into the Romanian diet was influenced by the Ottomans (Giurescu, 1966).

Currently, in our country, due to climate change and the dissemination of the benefits brought by the consumption of figs, more and more farmers are thinking of establishing fig orchards. However, fig trees are most often found in private gardens in the country (Stănică, 2017).

Research into the origin of the fig tree has placed it in western Asia, the Middle East, and certain areas of the eastern Mediterranean (from eastern Greece to Afghanistan).

Most fig varieties originate from *Ficus carica* L., a native species from Asia Minor (according to Mihăescu, 1977). Their exact number is, however, unknown due to problems of synonymy and homonymy (Saddoud et al., 2008).

It is considered that, in cultivation, there are over 1,000 fig varieties and biotypes adapted to the pedoclimatic conditions of different areas (Drobotă, 1986).

From a culinary and therapeutic point of view, the fig is a valuable plant. Figs are an important source of minerals and vitamins, lipids, phenols and enzymes (Hoza, 2000; Mitra, 1997),

proteins, amino acids, carbohydrates, but also easily assimilable sugars. The high medicinal value is given by the presence of phytochemicals (Naseer, 2021). Fig leaves also have a number of therapeutic properties and can be used in powder form in various mixtures or infusions.

Fig trees are sensitive to abiotic and biotic stress factors (Mostowfizadeh-Ghalamfarsa et al., 2022), which can affect fruit and leaf production. Among the well-documented and studied fig tree pests *Acaria fici* (fig mite), *Phryneta spinator* (fig beetle) and the nematodes *Hemicycliophora arenaria*, *Helicotylenchus multicinctus*, *Hoplolaimus* sp. and *Xiphinema* sp. (Faccoli et al., 2016; Kohl, 2011; Luc et al., 2005). The fig woolly aphid (*Ceroplastes rusci*), which feeds on the sap of the tissues, causing a general weakening of the plant, is also frequently reported.

An important viral disease reported worldwide is fig mosaic virus (Norman and Ali, 2013), which affects all fig varieties. Fig mosaic was initially reported in California, Egypt, Australia, and China, and has since spread worldwide through infected planting material and insect vectors. Fig mosaic is caused by numerous viruses, with three associated viroids (Preising, 2021, Jamous et al., 2020, Minafra et al., 2017) (Table 1).

Table 1. Fig viruses and viroids

No.	Virus name	Genus
1	*Fig leaf mottle-associated virus* 1 (FLMaV-1)	*Closterovirus*
2	*Fig leaf mottle-associated virus* 2 (FLMaV-2)	*Ampelovirus*
3	*Fig mosaic virus* (FMV)	*Emaravirus*
4	*Fig latent virus* 1 (FLV-1)	*Trichovirus*
5	*Arkansas fig closterovirus*-1 (AFCV-1)	*Closterovirus*
6	*Arkansas fig closterovirus*-2 (AFCV-2)	*Closterovirus*
7	*Fig mild mottle-associated virus* (FMMaV)	*Closterovirus*
8	*Fig cryptic virus* (FCV)	*Alphacriptovirus*
9	*Fig fleck-associated virus* (FFkaV)	*Maculavirus*
10	*Fig badnavirus* 1 (FBV-1)	*Badnavirus*
11	*Apple dimple fruit viroid* (ADFVd)	*Apscaviroid*
12	*Citrus exocortis viroid* (CEVd)	*Pospiviroid*
13	*Hop stunt viroid* (HSVd)	*Hostuviroid*

Source: Minafra et al., 2017, Jamous et al., 2020

Fig mosaic was first reported and described in the early 1930s in California by Condit and Horne (1933), and has spread to several fig-growing countries, including Turkey (Ülkümen et al., 1948; Özalp and Heper, 1972). The disease is not transmissible through latex or seeds (Martelli et al., 1993; Elbeaino et al., 2006), with Flock and Wallace reporting in 1955 that the virus is successfully transmitted by the eriophyid mite, *Aceria fici*, a monophagous mite that feeds on plants in the *Moraceae* family. The last reports of the presence of the mite were in the United States and Greece in 2023 (https://www.inaturalist.org).

Elbeaino et al. (2006) suggested that figs harbor a species of closterovirus, for which they proposed the name *Fig leaf mottle-associated virus* (FLMaV). The same closterovirus has also been reported in Tunisia (Nahdi et al., 2006). Recently, another representative of the *Closteroviridae* has been identified, and is called *Fig leaf mottle-associated virus* 2 (FLMaV-2).

The disease is transmitted mainly by vegetative propagation (cuttings) and by insect vectors, including the mites *Aceria ficus* and *Tetranychus urticae*.

Symptoms can be visible on leaves and fruits. Yellowish spots are visible on the leaves that contrast with the green tissue. Later, a rusty coloration appears at the edge of the yellow spots, caused by the death of epidermal or subepidermal cells. Leaf deformation, stunted growth, and yellowing of leaves are also present. Yellowing and deformation may occur on the same shoot with normal leaves. Yellow spots similar to those on the leaves, but less obvious, appear on the fruit. Attack on the fruit leads to premature fruit drop or the formation of small, few fruits.

Fungal and bacterial diseases are considered among the most dangerous for cultivated figs (Lin et al., 2018).

Fig leaves are susceptible to attack by fungal and bacterial pathogens, which cause various diseases and spots.

Fungal pathogens are responsible for rust, fusarium wilt, anthracnose and other leaf spots, root rot (Guillaumin et al., 1993; Johnstone and Jones, 1997; Kim et al., 2000; Sreenivasaprasad and Talhinhas, 2005; Droby et al., 2011; Norman and Ali, 2013; Raheb et al., 2014; Al-Sadi et al., 2015; Kumar and Khurana, 2015; Li et al., 2015; Miyake and Nagai, 2017; Lin et al., 2018).

Fungal infections can rapidly degrade fig wood (Lansky and Paavilainen, 2010).

Among the fungal pathogens, *Alternaria alternata, Cladosporium cladosporoides, Cylindrocladium scoparium* and *Cercospora fici* have been identified.

Bacterial pathogens can cause leaf spots, as *Pseudomonas syringae* pv. *syringae* (Ivanovic et al., 2018, Norman and Ali, 2013). Symptoms of the disease caused by *Pseudomonas syringae* pv. *syringae* appear as necrotic lesions that, within a few days of infection, become larger, coalesce and cover most of the leaf (Ivanovic et al., 2018). Leaf symptoms appear and develop after cold and rainy weather (Ivanovic et al., 2018).

Crown gall, caused by *Agrobacterium tumefaciens*, is considered one of the most important bacterial diseases of fig trees (Turechek, 2012).

Leaf spot caused by *Cercospora fici* is one of the most widespread diseases of figs (Yao et al., 2015). It was first reported in 1918 in Florida (Matz, 1918) and subsequently in Pakistan, Oman, Africa and China (Crous et al., 1991; Al-Sadi et al., 2015; Wu et al., 2015; Nayab and Akhtar, 2016). In the early stages, small, yellowish to brownish spots appear on the leaf. The lesion expands, becoming reddish-brown with dark brown, irregular edges. The attack is present on both leaves and fruits.

In Romania, fig trees have proven to have increased resistance to diseases and pests. However, in isolated cases, diseases that seriously affected the production and lifespan of an orchard have been reported since 1981.

In recent years, the appearance of some leaf diseases has been observed in fig trees in Romania, as well as an alarming increase in the number of cases of fig mosaic, one of the most widespread diseases affecting most fig varieties (Serrano, 2008).

The present study focuses on foliar and fruit diseases of figs in the collection of the Faculty of Horticulture, USAMV of Bucharest.

This study can provide important data regarding fig pathology in Romania, as there is little information related to fig diseases identification.

MATERIALS AND METHODS

The biological material studied was represented by over 60 fig genotypes from Romania, Croatia, Italy, Iraq and England, propagated in the greenhouse and planted in the experimental field of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest. Over 50 cuttings made from the Fiorone Rosso genotype and 50 plants from the Galben Mare genotype were also included in this study.

The study was carried out in 2023 and 2024. Monitoring of fig cuttings was carried out during April - September 2023.

Foliar and fruit pathologies were identified through visual observations. For fungal pathogens, a microscopic examination was performed on symptomatic or incubated plant tissues on PDA medium (Potato Dextrose Agar, Scharlau). The incidence (%) of Fig Mosaic Disease was calculated on Galben Mare genotype.

Cuttings fragments from the Fiorone Rosso genotype, with symptoms of wilting and rot were incubated for seven days on PDA medium at 22-24°C. Colonies characterisc of the genus *Fusarium* were detected. Pure cultures of *Fusarium* isolates were grown on PDA in Petri plates and after 7 days, the cultures were observed for shape and size of conidia and chlamydospores.

The *Fusarium* isolate was also identified based sequencing of ITS rDNA. The sequencing was carried out with the support of the University of La Laguna, Tenerife, within the CIPEV group. The sequence (677 nucleotides, Figure 1) was BLASTed against Genbank database by the National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov; accessed on May, 19, 2024) nucleotide database.

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CGCGCGWCGGCSTCTCCGCTTATTGATATGCACATCCGCAGGTTCACCTACGGAAGC
CTCCGCTTATTGATGTGCTCTTCCGTGCGMTCACCTACGGAAGGCTGCGGSGYGGCC
CTCACTGTTAKGTAAGTTAKCGCGCTGAGAGGGACCCAGATCACCASTGCCAGGCCC
GTTCCCCSGGAAGGGGGGGACGGAGGAAKCAAATGACCCTCTCCCATACCTGGCCG
CAACCGATTGGGTTGGAAAYGATTTGACTTTAGATATTTCGAGATACTGAATCCTGC
ATGTCCCATTACTTACCWCATTTCGCTGGGTGCTTYMACAAAGYCRAAGACAARAT
ATCTTCTGCAGTTARTTTTACTTCTCTTATTTCTCTGCSTTTTTCCCCTCAGAGAAATY
CGATAAATTCATTGTTTAAACTTCCCCCGCGGACGCCCGGACCATTACATGCCTTTTC
GCATTAAGCCCGCTTSTATCTTTTTTGTASYCAAAAGCCTGGATTGGGKAGCCCYAW
TWACTCTGTTCCCCCWAKAAAAAAYMTYTCTCTACRGAGGCASRKGTGTRTRKGAK
AAAAAAGAAAAGKGTGYRCATGCCCCCCARWKWWWAATAGACCCCCTATACCCC
CAATCTCTATATTAAYTMTATAGAGACTCTTCCGSGKGTTCCTCTASAAAGGG
```

Figure 1. BLASTed sequence - *Fusarium oxysporum*

RESULTS AND DISCUSSIONS

Results on foliar and fruit pathology

Both in 2023 and 2024, Fig Mosaic Disease (FMD) affected the fig trees included in this study.

The common macroscopic symptoms associated with FMD on leaves were yellow, diffuse spots (mosaic), deformation and chlorotic mottling as well as both combined; necrotic rust-coloured band developed along the border of the mosaic spot as a result of the death of epidermal cells;

leaf deformations, showing weak, asymmetrical growth, yellowing, necrotic spots; leaf wrinkles. Another observation concerned the fact that both leaves with symptoms and normal leaves were observed on the same branch.

On fruits, FMD was associated with yellow or necrotic spots. The range of symptoms observed and the multitude of viruses present suggest that fig mosaic may be caused by the synergistic action of several viruses, in co-infection.

The pathology associated with FMD is shown in Figures 2 and 3.

The frequency of plants from Galben Mare genotype with FMD was 96%, almost all 50 plants observed being affected.

Another fig leaf pathology was the presence of dark brown spots, initially discrete and limited to the main and secondary veins, later spreading over the lamina and coalescing, with finally irregular and more or less necrotic tissues (Figure 4). Microscopic examination revealed morphological characters characteristic of conidia and conidiophores of *Passalora trichophila* and molecular analysis of the isolate is ongoing.

Several species of the genus *Passalora* are involved in fig leaf spots: *Passalora bolleana* (*Pseudocercospora bolleana*), *P. ficina, P. trichophila* and *P. urostigmatis* (Singh et al., 2012). *Passalora trichophila* sp. *nov.* was identified on the leaves of *Ficus mysorensis* (*Moraceae*) in a subtropical forest in India (Singh et al., 2012).

From the spots developed on the leaves, isolates belonging to *Cladosporium* genus was isolated (Figure 5). *Cladosporium* sp. was observed on fruits, as well as *Aspergillus niger*. While *Cladosporium* on leaves can be a secondary invader or a cause of leaf spot, on fruit it leads to discoloration and spoilage (Figure 6). On green or ripening figs, *Cladosporium* can appear as small, olive-green specks that enlarge into sunken, yellowish-olive lesions. These spots may turn light brown to black.

Aspergillus niger is well known as black mold on fig fruits, causing fruit decay.

Results on fig cuttings pathology

The rooted fig cuttings of the Fiorone Rosso genotype were monitored until September 2023, when samples were taken for laboratory analysis. The samples were represented by cuttings with symptoms of wilting and rot (Figure 5).

Colonies developed in Petri dishes with incubated cuttings fragments were identified as belonging to *Fusarium* genus, based on macroscopic and microscopic examination (Figure 6). Pure and monospore cultures were obtained for molecular identification. Characterization of the fungal cultures based on morphology and sequencing of ITS rDNA revealed that our isolate belongs to *Fusarium oxysporum* species.

The species *Fusarium oxysporum* is known for the economic damage caused to horticultural crops, the pathogen being difficult to control. The characteristic symptoms are wilting, the presence of yellow areas between the main veins of the leaves and rotting of the roots and the base of the stem.

The pathogen is a known colonizer of soils and various substrates, surviving for many years in them or in plant debris in the form of mycelium or resistant spores - chlamydospores, in the absence of the host plant. It can also be present in soil debris on greenhouse materials, solar panels, irrigation pipes, mechanical equipment and other field materials. *Fusarium* fungi can cause a number of serious plant diseases, with *Fusarium* being one of the most important pathogens (Beccari, 2022; Leslie and Summerell, 2013).

There are few reports in the literature of the presence of *Fusarium* pathogens in fig trees.

In Romania, the disease was reported in 1984 (Drobotă, 1986). Jahén-Rivera et al. (2021) reported the presence of *Fusarium solani*, *Alternaria alternata* and *Pythium ultimum* isolates in rooting fig cuttings that showed slow growth, chlorosis, wilting, fallen leaves and rot on the stems and roots. Plants produced in greenhouses may exhibit stem and root rot, caused by a complex of pathogens from the genera *Fusarium, Rhizoctonia, Pythium* and *Phytophthora*, favored by a relative humidity of 80%, temperatures of 25°C and high densities (Beccari, 2022; García et al., 2008).

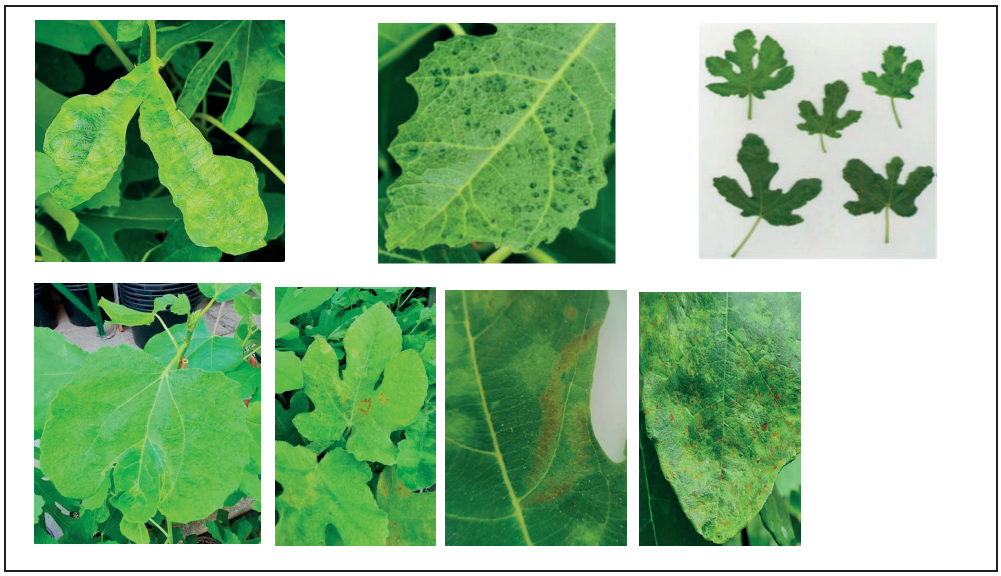


Figure 2. Deformation, embossing, discoloration, necrosis and combined leaf symptoms associated with fig mosaic disease (FMD)



Figure 3. Specific spots of fig mosaic disease (FMD) on leaves and fruits: irregular light green to yellow spots; fruits with yellow spots and necrotic spots



Figure 4. Leaf spotting - characteristic symptoms of *Passalora* spp.

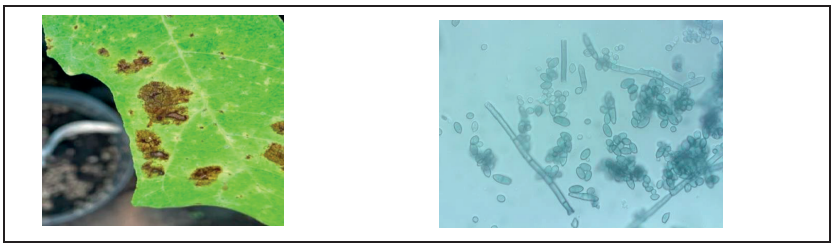


Figure 5. *Cladosporium* spp. on fig leaf



Figure 6. Characteristic symptoms of fruit rot caused by *Cladosporium* spp. and *Aspergillus niger*



Figure 7. Cuttings with wilt and basal rot symptoms

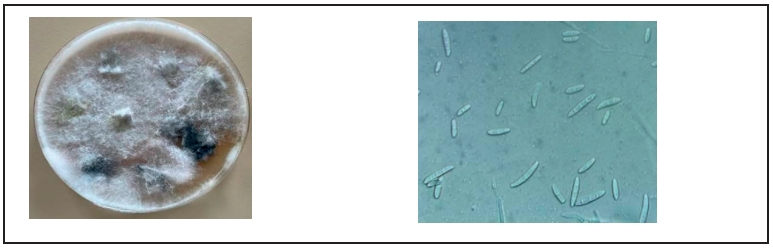


Figure 8. *Fusarium oxysporum* colonies and conidia

CONCLUSIONS

This study highlights the presence of Fig Mosaic Disease (FMD), *Passalora trichophila* (foliar brown spot), *Cladosporium* spp., and *Fusarium oxysporum* (wilt and basal rot) as a part of leaves pathology. On fig fruits, FMD, *Cladosporium* spp. and *Aspergillus niger* were detected and identified.

Characteristic symptoms of Fig Mosaic Disease (FMD) were detected on leaves and fruits. FMD is a complex disease associated with mixed virus infections, and different responses in symptom expression due to a specific virus or various virus combinations have been reported. Thus, the prevalence of those viruses remains to be analysed.

Also, another foliar pathogen, *Passalora trichophila* was detected on leaves and was responsible for brown spot disease.

In cuttings with wilting and basal rot symptoms the pathogen *Fusarium oxysporum* was detected and identified through morphological and molecular analysis.

The presence of *Cladosporium* spp. and *Aspergillus niger* was identified on fig fruits.

To our knowledge, this is the first time that *Fusarium oxysporum* and *Passalora trichopila* have been reported in Romania as important pathogens on fig leaves, completing the informations related o fig tree pathology.

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DYNAMICS OF CLIMATIC ACCIDENTS DURING PEACH, APRICOT, AND CHERRY DORMANCY AND FIRST GROWTH STAGES IN THE LAST 20 YEARS IN ROMANIA

Ivona Cristina MAZILU[1], Emil CHITU[1], Mirela CĂLINESCU[1], Florin PLĂIAŞU[1], Leinar SEPTAR[2], Zsolt JAKAB-ILYEFALVI[3], Sorina SIRBU[4], Calin-Viorel OLTENACU[5]

[1]Research Institute for Fruit Growing Piteşti - Mărăcineni, 402 Mărului Street, Mărăcineni, Argeş County, Romania
[2]Research Station for Fruit Growing Constanţa, 25 Pepinierei, Constanţa, Romania
[3]Research Station for Fruit Growing Bistriţa, 3 Drumul Dumitrei Nou, Bistriţa, Romania
[4]Research Station for Fruit Growing Iaşi, 3 Ion Vodă cel Viteaz, Iaşi, Romania
[5]Research Station for Fruit Growing Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: icmazilu@yahoo.com

Abstract

The paper analyzes the impact of climate change on the 20-year frequency of climatic accidents and the intensity of damage to apricot, peach, and sweet cherry flower buds in the continental climate of Romania. There was a rise from 2004 to 2023 in winter minimum temperature and mean temperature, especially in February, March, and April. Between 2004 and 2023, climatic accidents were registered in Romania in 14 years during the dormancy or in the first growth stages, i.e. damages caused by late frosts in 9 years, in three years (2006, 2010, and 2012) frosts below the hardiness limit of some fruit species causing damages during dormancy, and also in three years (2004, 2015, and 2016) bud damages caused by thermal oscillation in the dormant season (35-40°C in only 2-7 days). In 7 of the 20 analyzed years, the damages caused to the flower buds of some cultivars were major. Although until 2016, damages caused by frosts and thermal fluctuations in the dormancy season prevailed, since 2017, in six out of seven years, the damages were caused only by late frosts.

Key words*: winter minimum temperature, late frost, thermal winter oscillations, flower buds.*

INTRODUCTION

Since the climate strongly restricts fruit species' distribution and productivity (Chitu et al., 2016), plants have developed a series of strategies to adapt and deal with less favourable or even adverse environments over time. Such adaptation in the case of temperate species was the temporal distribution of development and growth phases, under the control of environmental factors such as photoperiodicity, solar radiation intensity, temperature, soil, and water (Sherman & Beckman, 2003). Among them, temperature controlled physiological processes during the vegetative season, dormancy during the winter, and vegetation onset (Łysiak & Szot, 2023).

Changing world patterns of climate, with seasonally uneven warming reported in Europe, characterized by winter and spring months warming twice faster compared to summer or autumn months (Zohner & Renner, 2019), could results in a redistribution of fruit species, but also, through phenology shifting (Zohner & Renner, 2019). The resizing of the vegetative growth season may expose them to certain conditions of stress/climatic accidents with repercussions on yield quality.

Global warming had different and even opposite consequences depending on the geographical area (Kopecka et al., 2023), from the increase of thermal and radiative stress, the insufficient accumulation of cold hours during dormancy, followed by the start of uneven vegetation, or contrarily, more rapid chilling accumulation correlated to early vegetation onset, the increase (or sometimes the reduction) in the frequency and intensity of climatic accidents such as early autumn frosts or, on the contrary, frosts late spring (Zahradníček et al., 2023).

In temperate zones, under the influence of environmental signals, fruit species enter

dormancy, when cold hardiness develops through gradual exposure to freezing temperatures as a plant's strategy to protect its meristematic tissues (Hermawaty et al., 2024). Depending on the moment in the rest period and exposure to freezing temperatures, the tissues of the trunk, branches, and flower buds acquired different degrees of cold hardiness, and the heating episodes during the winter could result in dormancy offset (Moran & Ginakes, 2024). During warm spells, cambium was found to be the most vulnerable to loss of cold hardiness (especially in S or SW trunk exposures), and almost as sensitive was the phloem. At the same time, xylem damage was very rarely reported (Moran et al., 2021). Endo-dormant floral buds had the highest cold hardiness at the opposite pole (Moran & Ginakes, 2024).

Therefore, a time characterized by higher-than-normal temperatures during winter could stimulate the activity of some plant tissues, leading to their hydration and loss of hardiness (Chitu et al., 2014). In these conditions, the subsequent decrease in temperature, or rather the restoration of normal climatic conditions, led to serious consequences because, if floral buds damage was only reflected on the fruit yield of the year in which it occurs, damage to the trunk, even if it was not severe enough to kill the trees, could have long-term effects (Sheppard et al., 2016) as once a tree was damaged, wounds were vulnerable to fungal and insect attack (Houghton et al., 2024).

Secondly, the milder winters lately have created the conditions for the species' need for cold to be fulfilled as early as December in the last decade. After completing the necessary cold (endo-dormancy), the plants enter eco-dormancy, which is controlled by the air temperature. In this phase, at least six consecutive days with temperatures above the basal limit of the phenophase, which is species/cultivar-specific, were required for vegetation onset (Łysiak & Szot, 2023).

The sensitivity to negative temperature of fruit species increased with the advancement of the phenological stages, and late frosts were frequently recorded during the bud-break period or flowering. The intensity of the damage resulted from the intersection between the level of sensitivity to negative temperatures of the floral organs and the size of the reduction in air temperature below their frost resistance limit. Regarding the reproductive structures, stigma, style, and the flower stalk/peduncle in high mountain plants were the most frost-susceptible (Neuner et al., 2013).

Sweet cherry occupied the third place (as surface) in Romania, after apple and plum, while apricot and peach were placed in the 5th and 6th positions (unpublished data). Sweet cherry frequently found favourable conditions except for the mountainous areas, especially in the NE, SE, and S areas. The maximum thermal suitability for the peach was concentrated in the SE and S, and the apricot encountered in the NE, E, SE, and S even more favourable conditions than sweet cherry and peach. Previous studies indicated that in Romania, orchards frequently dealt with late spring frosts, and their effects became more and more severe due to the spring phenological advance (Chitu et al., 2016; Florea et al., 2019). Starting from these observations, the present study aims to analyse the damage recorded in Romania's peach, apricot, and sweet cherry orchards during temperature deviation from normal in the winter-spring period.

MATERIALS AND METHODS

The study was carried out in the experimental field of Research Institute for Fruit Growing Pitesti (RIFG), Arges county (Maracineni, Southern part of Romania, 44°53’56” N, 24°51’35” E) and in other eight Research Stations and orchards located in Baneasa (Bucharest, in S-SE, in the Romanian Plain; 44°30’05” N, 26°04’13” E) and Moara Domneasca (44°29’45” N, 26°15’09” E), Constanta (Valul lui Traian, in South-East; 44°10’03” N, 28°29’31” E), Bistrita (in North, in the Transylvania Plateau; 47°10’01” N, 24°29’50” E), Iasi (in North-East; 47°13’29” N, 27°09’41” E), Dabuleni (Dolj, in South; 43°47’48” N, 24°03’47” E), Valcea (in the hilly area of the Southern Carpathians, 45°08’26” N 24°22’01” E), Voinesti (Dambovita, in the Southern area of the Southern Carpathians, 45°04’58” N, 25°14’22” E), Targu Jiu (Gorj, in the area of the Getic Subcarpathians; 45°02’26” N, 23°18’58” E), Falticeni (Suceava, in North-East; 47°27’08” N, 26°17’39” E), and Cenad-Timis (in East; 46°06’03” N, 20°35’35” E).

A phenological stage database consisting of observations made for sweet cherry, peach, and apricot cultivars grown in the above-mentioned field trials and in different orchards from Romania was used, i.e., bud swelling (51 BBCH), bud burst (53 BBCH), beginning of flowering (61 BBCH), end of flowering (69 BBCH), and young fruit (71 BBCH).

For temperature dynamics, the weather data were determined in a fenced meteorological platform, 25 m × 25 m, at 44º53'53.42'' North, 24º52'03.55'' East, and an altitude of 287 m a.s.l, during 2004-2023, from an automatic weather station, WatchDog 900ET, from Spectrum Technologies Inc., USA. From December 1 to May 31, the weather variable used was air temperature, which was measured at 10-minute intervals by sensors in the automatic weather station.

Starting from the multiannual (1969-2022) database, temperatures (minimum, maximum, and mean) multiannual averages in the Pitesti-Maracineni area were calculated, and their evolution was studied starting from 2004 for the next 20 years. Winter low temperatures and late frost events, especially those preceded by an unusual increase in temperature related to trees' phenology and frequency, were presented. To appreciate the low temperature impact intensity on sweet cherry, peach, and apricot, orchard and laboratory observations were performed, i.e., damages on S or SW exposure of the trunk and of the fruit buds, flowers, and young fruits, following the protocol proposed by Larsen (2009). The negative critical temperatures for fruit species, below which damage to the fruit buds/flowers/fruits was considered to occur, were those presented by Muray's (2020) study.

Linear regression equations and their statistical coefficients have been calculated for temperatures and phenological data with MS Office EXCEL and IBM SPSS Statistics version 20 software. Therefore, the trend of the decadal averages of the minimum and maximum temperatures, as well as the differences between the maximum and minimum temperatures of two successive pentads during the 2004-2023 winter and spring months and the evolution of the species' first four phenological stages, were presented and discussed.

RESULTS AND DISCUSSIONS

Temperature trends 2004-2023

To observe the amplitude and frequency of temperature oscillations during the wintertime in the Pitesti-Maracineni area, the decadal trends of the minimum and maximum temperatures in the December-May interval were analysed (Figure 1). As presented, there were wide oscillations, especially of the maximum temperature trends between December and the second decade of March, followed by a much lower value of trends until the end of May. The maximum temperature showed an increasing trend in most of the analysed decades, interrupted by a decrease in the first decade of December and stagnation or a very slight decrease in the second decade of January, but also after the second decade of March. Significant is the upward trend of 3.2°C per decade in mid-February. This was an early warning signal because rising temperatures can stimulate the vegetation onset, especially with the warm end of February and the beginning of March, or it could just activate the cambium, exposing the trunk of the trees to further normal low temperatures during winter.

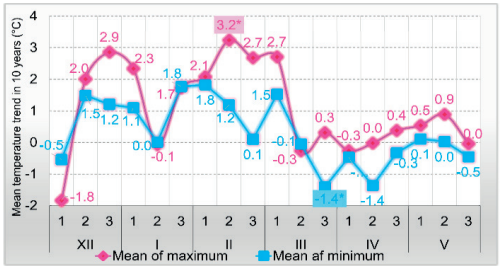


Figure 1. Decadal trends of minimum and maximum temperatures in Pitesti-Arges (Maracineni) in the December-May interval (2004-2023)

The minimum temperature oscillations were smaller than the maximum temperature in the first half of the analysed interval. The minimum temperature also increased in December, January, February, and the first decade of March. However, a slight cooling tendency was still observed in early December, followed by stagnation in January's second decade and March's third decade.

This warming trend may contribute to the earlier accumulation of cold hours during deep dormancy, making the species sensitive to subsequent temperature increases above the basal threshold. Thus, the onset of vegetation could occur. The consequences are fruit bud damage, especially since, as the graph indicates, in the third decade of March there is a significant decrease in air minimum temperature of 1.4°C per decade, followed by a second minimum temperature decrease in mid-April, when the sweet cherry are usually at least in bloom, peach at BBCH 69, and apricot even more advanced.

Increases in maximum temperature during the winter in conditions of a stagnant minimum (if not even in a slight decrease) could reduce the frost resistance of the trees and have more serious effects, as the thermal amplitudes can even reach 35-40°C in Pitesti - Arges (Maracineni). As a result of maximum and minimum temperatures trends (sometimes even opposite), a significant increase in the frequency and amplitude of thermal oscillations occurred in the analysed December-February period.

The warming of the winter (by increasing the maximum temperature in February and early March), determined a general tendency of earlier occurrence of flower and fruit development phenology accentuated for early stages (especially BBCH 51) and more temperate (with a small and insignificant slope) for the beginning and end of flowering (BBCH 61 and 69).

In sweet cherry ('Gemersdorf' cultivar, Figure 2), a significant advance of almost 10 days per decade was registered for vegetation onset (BBCH 51).

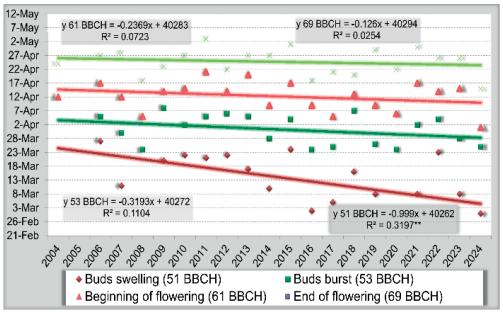


Figure 2. Linear relationships between the time to onset date (TOD) of the four phenology stages and the year of study for the 'Germersdorf' sweet cherry cv. (Pitesti, 2004-2024)

At the same time, bud breaking, beginning and end of flowering were not significantly delayed, and the advance recorded for the three phenophases was low (three days, 2 days, and one day, respectively).

For peach ('Filip' cv., Figure 3) a greater phenological advance was registered compared to the cherry, very significant for BBCH 51 (almost 12 days per decade), distinctly significant in the case of BBCH 53 (10 days per decade) and also very significant for BBCH 69 (9 days per decade). Still, the beginning of flowering (BBCH 61) showed a non-significant tendency to produce earlier by 5 days per decade, probably because this thermophilic species finds unfavourable conditions in the second decade of March, while in April it is still uncertain from a climatic point of view.

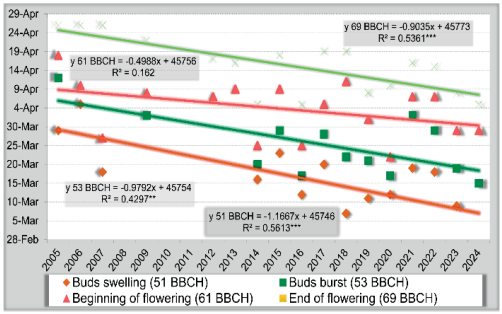


Figure 3. Linear relationships between the time to onset date (TOD) of the four phenology stages and the year of study for 'Filip' peach cv. (Constanta, 2005-2024)

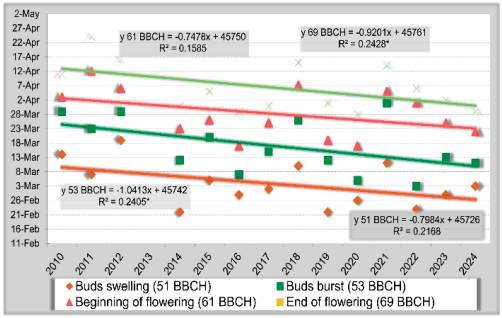


Figure 4. Linear relationships between the time to onset date (TOD) of the four phenology stages and the year of study for 'Elmar' apricot cv. (Constanta, 2010-2024)

The trends of the four phenological phases of the apricot (Figure 4) are somewhat similar. However, their statistical significance differs, as only BBCH 53 and 69 showed significant

advances of 10 and 9 days, respectively. Although there was a trend towards the earlier occurrence of BBCH 51 and 61, on the background of temperatures with wide oscillations from one year to another, the advance of 8 and 7 days was insignificant, respectively.

Analysis of climatic accidents from 2004 to 2023

2004

In 2004, the highest mean air temperature in the second pentad of the last 35 years was recorded (12.9°C, February 6), when the maximum air temperature reached 18.3°C on February 6 and 18.6°C on February 7. These very high temperatures, although they did not cause bud swelling, contributed to a decrease in the trees' frost resistance. After only 6-7 days, on February 13, the average air temperatures dropped to -13.4°C (0.3% probability to register a lower temperature in the third pentad). The minimum temperature decreased to -21.7°C (0.1% probability to register a lower temperature). As a result of these exceptional phenomena, the fruit buds of peach and apricot froze (99.5% and 99.8% damage, respectively). Peach vegetative buds also froze at the top of the crown (53%) but mostly at the base of the crown (100%) (Chitu et al., 2004).

2006

January 2006 differed significantly from the previous months by very low temperature, close to the absolute minimum of the area (-24.4°C, recorded on January 13 and 17, 1985). The coldest interval was January 24-26, when the minimum temperature dropped below -20°C. On the 24th, -23.3°C was recorded in the air, the second lowest value after the station's absolute minimum of -24.4°C. If the absolute minimum occurred in the middle of the month, the lowest minimum air temperatures since January 2006 were recorded in the third decade. Therefore, the air temperature of -23.3°C was the lowest value in the third decade of January in the last 38 years (1969-2006) and led to 83% flower bud damage in sweet cherry (Budan et al., 2006).

2008

In 2008, after high temperatures in February and March, which hastened the vegetation onset, very low minimum temperatures appeared in the sixth pentad of March, culminating with -5.6°C in the morning of March 27. The sudden drop in the minimum air temperature occurred after three days with very high average and maximum temperatures (daily P≤85%) for this period (the maximum on March 23 was 19°C, on the 24th 19.1°C and the 25th 17.6°C), with intense sunshine and very low atmospheric humidity of 34-35% (daily P≤3-4%). Although sweet cherry was not in bloom (advanced bud breaking, 55 BBCH single flower buds visible, still closed, borne on short stalks, green scales slightly open), the ovules were affected (Chitu et al., 2010), and up to 96% of sweet cherry yield was compromised in Pitesti.

2009

As a result of very low temperatures (-3.4 to -6.0°C) in the last decade of April 2009, in many areas of the country, damages caused by late frosts on flowers, young fruits, and shoots of most fruit tree species were observed. Thus, after March 30, in Pitesti-Arges (Maracineni), the maximum air temperature rose to over 15°C in 21 days out of 24 and to over 20°C in 11 days, with a maximum of 23.9°C. In the same interval, the minimum temperatures did not drop below 1.2°C; in 9 days out of 24, they were higher than 6°C.

On the night of April 23 in Pitesti-Arges (Maracineni), although the temperature in the weather shelter only dropped to -1.7°C, outside the shelter, at the frost sensors, it decreased to -3.4°C (at 2 m from the ground) on dry bulb and to -4.2°C on a wet bulb. Sweet cherry flowers (falling petals, 66 BBCH) were massively affected (100%). In Baneasa-Bucharest (-2°C air temperature, -4°C soil surface temperature), damage to young fruits (70 BBCH) reached 40% in cherry, 70% in peach, and even 95% in apricot. In Constanta (-3°C in the air and -6°C on the surface of the soil), damage to young fruits (70 BBCH) in sweet cherry rose to 35%, in peach it was 85%, while apricot was affected in a slightly lower proportion of 80%. In Falticeni-Suceava (-2.5°C in the air and -4.2°C on the ground), the damage recorded to the sweet cherry (67 BBCH) was 48%.

2010

In Pitesti-Arges (Maracineni), after a first half of January 2010 warmer than normal (minimum

temperature probabilities of up to 95% and in 9 days out of 20 of over 80%), starting from January 21, temperatures dropped suddenly. They remained at this level for 8 days. The coldest period was reported on the 25th at 5 a.m., when the temperature outside the meteorological shield dropped to -23.9°C, being only 0.5°C higher than the absolute minimum of the station, -24.4°C, and in the shelter to -22.6°C. On January 25 and 26, the air temperature outside the meteorological shelter remained below -20°C for almost 24 hours. Under these conditions, the losses in the sweet cherry orchard (00 BBCH, Figure 5) rose to 73% (Budan et al., 2010).



Figure 5. Low winter temperature damages the tree trunk and flower buds (2020)

2012

As a result of very low temperatures in January and February 2012, frost damage to the vegetative and generative organs of tree species was reported in many areas of the country. In Pitesti-Arges (Maracineni), the lowest air temperatures were recorded in a short period: the first cold wave between January 29 and February 2 (with minimums between -17.5 and -20.1°C) and the second between February 9 and 11, 2012, with even lower temperatures (minimums between -18.1 and -23.4°C), while the temperature recorded at the surface of the snow layer dropped down to -27.1°C. Among the 9 locations analysed, the highest temperatures in the two intervals were recorded in Constanta (-17.4°C and -17.6°C), and the lowest in the second cold wave in Dabuleni-Dolj and Falticeni-Suceava (-27°C).

The calculation of the probability of occurrence of very low air temperatures in February in Pitesti-Arges (Maracineni) indicated that the value of -23.4°C represented the lowest temperature recorded in the last 44 years (the absolute minimum of February so far was -22.1°C, recorded on February 14, 1985). Phenologically, the species had accumulated the necessary chilling and were going through eco-dormancy. However, even under these conditions, the damage produced at Pitesti by the low temperatures reached the maximum threshold of 100% for some sweet cherry and peach cultivars. Damage was recorded in the orchard by affected fruit buds of up to 100% in cherry, 46.4% in apricot, and especially in peach, where the losses were (100%).

The frosts at the end of January and the first decade of February 2012 caused the greatest damage to peach and apricot species in the Dabuleni-Dolj area (minimums of -24.3°C on January 31 and -27.0°C on February 1) by freezing all fruit buds and annual branches. In Baneasa-Bucharest (minimum of -22°C on January 29 and -24°C on February 9), for the same species, flower buds were affected in maximum percentages of 92%, and the damage to the annual branches rose to 35%.

2014

Due to the warming trend of November 2014, which favoured the accumulation of more than 900 chilling hours (between 0°C and 7°C) by the end of December, severe tree stunting may have occurred at the end of December 2014. In the last decade of December 2014, the minimum negative temperatures of the day varied between -0.6°C and -5.0°C, with the absolute minimum of -20°C on December 31. Even if the negative temperatures were maintained in the first decade of January **2015**, reaching the absolute minimum of -19.6°C on January 8, they did not affect the flower buds, while the species were in deep rest on that date. Warm periods in December, followed by a drop in temperature at the end of December and beginning of January, were also recorded in other regions of the country. However, no damage was reported, as the trees were still in vegetative rest.

In the southeast of the country, in Constanta, the minimums of -10.4°C in the third decade of December 2014 and -16.1°C in the first decade of January 2015 were preceded by maxima of 12.2°C and 16.0°C in the last two decades of December 2014. Under these conditions, damage that reached 14% in apricot and 15% in the peach was recorded (both species being at that time in the early phenophase of bud swelling, 51 BBCH).

2016

2016 started after a warm autumn, with maximums of up to 22.1°C. The first and last decade of December 2015 were characterized by high temperatures, with maximums of up to 16.4°C and 19.7°C, respectively, and were followed by a sudden drop in temperature to minimum values from -10.1°C to -14.9°C between 29 December 2015 and 5 January 2016. At the end of December, over 900 chilling hours had been accumulated, so that between January 11 and 14, a slight warming with maximums of 9.1-13.8°C, followed by a second wave of low temperatures between -11.8°C and -17.3°C between January 17 and 25, made the trees strongly susceptible. Maximum damage recorded for eco-dormant species rose to 35% in peach, 50% in apricot, and 73% in cherry.

Subsequently, the warm February 2nd and 3rd decades (with maximum rising to 22.2°C) and the first March decade (with a maximum of 17.3°C) were followed by a thermal decline on March 17 to -4.2°C. Since phenological, at that time the peach and apricot were at the stage of pink petals, and the cherry at bud breaking (53 BBCH), following that frost episode, additional damages of 35% were recorded for the cherry, 60% for the peach, and 95% for the apricot.

2017

In Pitesti-Arges (Maracineni), the low temperatures of January 2017 (negative average and minimum values down to -21.3°C) and the close-to-normal February from a thermal point of view were followed by a marked warming in March (with maximums up to 24°C) and in the first half of April (maximum of 22.6°C), which determined a phenological advance of the species and exposed them as a result of the effects of frost late from the last pentad of April. The phenological stage of the three species, young fruit, made them susceptible to damage in a percentage of 10% at the drop in temperature starting from the threshold of -2.2°C and 90% at 3.9°C.

The first exposure was on April 21, when temperatures were below -2.2°C (the 10% damage threshold) and the absolute minimum dropped to -3.2°C. The next exposure to critical temperatures for the species was on April 22, when the minimum reached -4.2°C (outside the radiation shield), exceeding the 90% damage threshold (3.9°C).

Sweet cherry, in a very advanced phenological phase of young fruit (70 BBCH), was very intensely affected in Voinesti-Dambovita (-4.3ºC, 95% frozen fruits) and in Pitesti-Arges (Maracineni, Figure 6), where the percentage of damaged fruits rose to 91%, but very little in Baneasa-Bucharest (-1°C) and Iasi (-0.5°C), except 'Regina' cv. with 85% affected flowers. The damage recorded in the peach was also very high due to the high sensitivity of young fruit to frosts. In Valcea, peach yield was totally compromised, and in Pitesti-Arges (Maracineni), the damage was lower (84%). Without data from Constanta, the lowest damages were registered in Iasi (65%). In the same phenophase of young fruit (70 BBCH), the apricot was totally affected in Voinesti-Dambovita and Valcea. Apricot damages rose to 80% in Baneasa and exceeded 85% in Iasi.



Figure 6. Ice on flowers and young fruits, in the morning of April 21, 2017, in Pitesti-Arges (Maracineni)

2018

In 2018, the interval with very high temperatures (average, maximum, and minimum) that started on the first days of February was interrupted for 5 days between February 25 and March 1, when the lowest minimum temperatures of the winter 2017-2018 were reported. In these circumstances, a single climate accident with significant negative effects from the beginning of the growing season was recorded. In Pitesti-Arges (Maracineni), on March 1, a record minimum temperature for March in the last 50 years of climatological observations was registered, i.e., -19.8ºC (-24ºC outside the radiation shield), a temperature by 0.3ºC lower than the absolute minimum of the month (1969-2017).

The greatest damage recorded during this period occurred in the apricot and peach orchards, which were in the phenophase of bud swelling (51 BBCH), respectively, at the onset of bud swelling, and in the cherry, which, similar to the peach, was also at the onset of bud swelling.
The damage reported in the northeast of the country, in Iasi, for apricot rose to 85% of fruit buds affected, while for peach the damage was only 20%. In the southeast, in Constanta, as a result of the frost on March 1 (-13.3ºC) and the glaze ice (-5.7ºC) on March 19-20, fruit bud damages of 30% in peach and 52% in apricot were found. In the south, at Dabuleni-Dolj, the percentages of fruit buds affected by the frosts between February 27 and March 2 (-17.6ºC) rose to 80% for apricot and 10% for peach. In Pitesti-Arges (Maracineni, -24ºC outside the radiation shield on March 1), for peach, the maximum percentage of affected fruit buds was 59%, and for sweet-cherry it reached 42% due to the frost.

2019

In 2019, the warm period in February (with highs rising to 19.2ºC) and March (highs of 23.6ºC) favoured a phenological advance in both March and April, months in which three episodes of late frost occurred.
The first such event was recorded on March 28, when in Pitesti and Constanta the peach and cherry were in the budding phenophase (53 BBCH, -3.9ºC), and the apricot in the flowering phase (BBCH 65, -2.7ºC). The very low temperatures since the morning of March 28, 2019 (below -5ºC, and even -7.3ºC in Targu Mures and -6.4ºC in Jimbolia), recorded in other localities in Transilvania, but also in Banat, which fell below the critical temperatures of the phenophases experienced by the species, could affect, locally, the flowers of the apricot species and the peach and cherry fruit buds. On March 29, the second event had a lower impact on the trees, with the recorded minimums dropping only to -4.9°C (at Targu Mures).
The subsequent increase in temperature favoured the phenological advance. Thus, in the first week of April, in Constanta, the peach and the cherry were in the phenophase of flowering (63 BBCH), and the apricot was going through the end of flowering (69 BBCH). Between April 3 and 7, very low minimum temperatures were recorded in Constanta, from -4.1ºC on April 3, to -5.1ºC in the morning of April 6. Under these conditions, damages of 96% to peach flowers were estimated. In apricot, the damage was maximum (with 100% frozen flowers), and in cherry, 65% of the flowers were affected by frost.
Temperatures below the critical thresholds of the current phenophase were recorded in the morning of April 4 in other localities in the south-eastern area of Romania (especially in Tulcea -4.3ºC and Grivita -4.1ºC).

2020

The first months of 2020 were characterized by above-normal average temperatures, especially February and March. This warm period stimulated the start of vegetation and determined a phenological advance of the species (14 days for cherry in the Pitesti area). In the South and East of Romania, starting on March 16 and ending on April 7, there were five waves of late frosts with temperatures below the minimum critical thresholds (10% damage) of the current phenophases, for almost all fruit tree species. In many cases, even critical thresholds were reached for damage of more than 90% of floral organs. The event was seconded in the south and east of Romania, by a drop in temperatures in March to -7.1°C, followed in April by a thermal decline to -3.9°C.
In the first such frost (March 16-17), the temperatures dropped below the threshold of 10% damage to the cherry (53 BBCH) and to the peach in the pink button phenophase (57 BBCH), even exceeding the temperature of -4.4°C (the thermal threshold for 10% damage to the apricot in the white button stage, 57 BBCH). The minimum temperature exceeded -6.2ºC (March 16), dropping to -7.1ºC on March 17.
The damage caused by the first wave of late frosts on March 16 and 17 was accentuated by the fact that they followed two weeks of exceptionally high temperatures (higher than 90% probabilities on 9 days out of 14. During this interval the highs culminated in 23.3ºC on March 13 (99% probability, higher values occurring only once in 100 of years), so that after 3 days the temperatures drop to -6.2ºC and the next day to -7.1ºC (3% probability, lower

values being registered only once every 33 years), the thermal oscillation being 30.4ºC.
The warm period from March until March 21, between March 26 and 30, and daily highs of up to 17.1 ºC, favoured the phenological evolution. On top of these conditions, during the first 7 days of April, there was a period with minimums lower than the damage thresholds due to late frosts in the three species. Thus, on April 1, the minimum dropped to -5ºC (2% probability, lower values once every 50 years), on April 3 to -4.2ºC (≤3% probability), and on April 7 to -4.0ºC (≤2% probability).
The peach from Pitesti in the pink button phenophase in mid-March and flowering between April 1 and 7 was insignificantly affected (2-3%) by the low temperatures. The cherry tree, very advanced phenologically, 53 BBCH in the middle of March (2 weeks earlier than the multiannual average), when in standard years the flower buds were barely swollen and at the beginning of flowering on April 7 (April 15 being the average date of this phenophase), recorded heavy damage of more than 90% affected flowers.
In Iasi (-4.9ºC on March 16 and -3.0ºC on March 17, 95.5% damage was reported to the apricot in full bloom (65 BBCH), 39.5% to the peach (pink button), and 56.7% to the cherry (swelling buds, 51 BBCH). In Falticeni-Suceava (-5.0ºC on March 16, respectively -3.0ºC on March 17), damage to the cherry species (bud swelling, 51 BBCH) was 15.0%. In Voinești-Dambovita (-2.3ºC on March 16 and -4.3ºC on March 17), 20.0% damage was reported to cherry (swelling buds), 95% to apricot (full bloom), and 25% to peach (beginning to bloom). In Bistrita, the minimum temperature of -3.8ºC recorded on the night of April 15 - 16 was below the threshold for damage to fruit buds, and major damage was recorded to cherry trees (100%).

2022

The warm period in the first two months of 2022 (average temperature of 3°C above normal in January and 4.6°C in February) preceded the temperature drop in March to lows of -9.3°C and then -3.8°C in April. A first frost episode occurred between March 9 and 20, which culminated with lows of -9.3°C (March 12), exceeding the 10% damage threshold for cherry (in the advanced swelling phenophase) and peach (red calyx) as well as the 90% damage threshold for peach (pink button) and apricot (white button).
Between March 21 and April 3, there followed a similarly warm period, with maximum temperatures between 11.8 and 22.1°C, followed by the second episode of frost, which consisted of a drop in temperatures on April 4 and 5 to a minimum of -3.8°C, when the phenophases passed were white button for cherry and beginning of flowering in the case of peach and apricot, the species being therefore susceptible to damage in proportion to 10%.
A third wave of high temperatures was recorded between April 6 and 17 (with maximums between 10.8°C and 24.1°C in the Maracineni Arges area), followed by the third episode of frost, in which there was a drop in temperature between April 18 and 20, in the phenophase of petal fall in cherry and young fruit in peach and apricot. This episode was reported only in certain regions of the country. -2.0°C was reported in Bistrita and Iasi on April 19, which did not cause damage. In Botosani, -5.8°C was recorded on April 18 and -3.0°C on April 20, with damage of 40% to the cherry in full bloom and 50% to the peach and apricot in the young fruit stage.
In Pitesti-Arges (Maracineni), the losses of fruit buds/flowers in the cherry species were 52% due to the first two periods of frost. In Baneasa, at the Baneasa Experimental Base, the losses were 30% in apricot (young fruit) and 55% in cherry (petal fall). At the Moara Domneasca Experimental Base, the losses were 80% for apricot (young fruit), 28% for peach (young fruit), and 12% for cherry (full bloom). In Iasi (-2.0°C), the losses were 40% for cherry (full bloom), 50% for apricot and peach (young fruit). In the southern area of Botosani County and the northern area of Iasi County (-5.8°C), cherry losses (full bloom) were maximum (100%). At Targu Jiu (-3.0°C on April 21), it affected the cherry (full bloom) insignificantly. In Voinesti (-1.8°C), the loss of flowers was 25% for cherry (the beginning of flowering). In Bistrita, no damage was recorded under a minimum of -2.0 degrees for the same species (cherry) (white bud and beginning of flowering).

2023

2023 started very warm (average monthly temperature almost 5°C higher than usual). Moreover, increases in the average temperature were also recorded in the other months that preceded the flowering. Except the warming in the first eight days of January (with maximum between 10.4°C and 20.3°C), and the 18-21 January interval (maximum of 16.2°C), temperatures favourable for the vegetation onset (over 10°C) were recorded starting from the second half of February almost every day, simultaneously with the decline of the negative temperatures. In March, in 24 of the first 30 days, the maximum temperature was 10.3-21.7°C, encouraging the start of the vegetation.

By lowering the minimums on March 30 to -6°C (temperature measured outside the radiation shield) and the persistence for about an hour of temperatures below -5.5°C, the limit temperatures for 10% damage to cherry trees in advanced swelling, and even the limit temperature for a percentage of 90% damage to flowering peach, were exceeded. In these conditions, relative to the limit of frost resistance of the fruit organs (Muray, 2020), the apricot, at the petals fall, was even more affected than the peach and sweet cherry. In Pitesti-Arges (Maracineni), fruit bud/flower losses rose over 85% in the sweet-cherry orchard (61 BBCH) and peach (65 BBCH-full bloom).

In Baneasa-Bucharest (-5.0°C on March 30) the damage was maximum on sweet-cherry (BBCH 61) and apricot, and when exposed to -5.3°C (Moara Domneasca-Bucharest), the cherry lost 50% of its buds (green bud), the peach 90% (65 BBCH full bloom), and the apricot 100%. In Bistrița (-4.3°C/March 29, -5.9°C/March 30), cherry losses were 80% (green button). In Constanța (-4.0 degrees/March 30), damage to cherry (white button) was 40%, to peach (blooming) 64%, and to apricot (petal shaking) reached 72%. In Iasi (-2.0 degrees/March 28 and 29), damage was lower for peach (16%, at flowering), compared to apricot (30% at the end of flowering). In Voinesti-Dambovita (-6.8 degrees/March 30), 25% of the cherry buds were affected (advanced swelling/bud breaking). In the east of the country (Cenad-Timis, -4.2 degrees dry bulb/-5.6 degrees Wet bulb), the damage rose to 50% for cherry (flowering onset), and in peach and apricot, the losses were maximum (full bloom).

CONCLUSIONS

The impact of climate change on 20 frequencies of climatic accidents and damage intensity to apricot, peach, and sweet cherry flower buds in the continental climate of Romania was analysed. In 20 years of study, 14 climate accidents occurred, all preceded by warmer periods compared to normal. Out of the 14 events discussed, nine were damages caused by late frosts. Six occurred during the dormancy period: three (2006, 2010, and 2012) of them were frosts below the hardiness limit of some fruit tree species causing damages during dormancy and in other three (2004, 2015, and 2016) bud damages were reported, caused in the dormant season by very high thermal oscillation (of 35-40°C) in only 2-7 days. Among the six accidents during the dormancy (4 in January and 2 in February), in five the air temperature dropped below -20 degrees, and in 3 it was even lower than -23 (and one of them occurred in February, when -27 degrees were recorded on the snow surface). Two of the winter low temperatures were preceded by a rapid temperature drop of 39.2°C in 7 days (2015) and 34.6°C in 3 days (2016). Of the 9 late frosts, seven occurred in March (the lowest temperature was -20.4°C/March 1, 2018, 51 BBCH) and five in April (with a minimum of -5.8°C/April 18-21, 2022). The most damaging event was the late frost of April 21-22, 2017 (70 BBCH), when fruit yield was compromised for all three studied species. An alarm signal could be raised because there was a statistically guaranteed trend of warming in February (and therefore earlier stimulation of vegetation onset). However, the temperature remained the same in the following two months or decreased slightly. Therefore, the danger of late frosts will increase in the future in Romania, which requires the judicious choice of species and cultivars suitable for each pedoclimatic condition and the timely adoption of adequate frost protection measures.

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PHOSPHORUS AND IRON CONTENT IN APPLES IN DIFFERENT SALES CHANNELS

Marko PETEK[1], Matilda POVODNIK[2], Tomislav KARAŽIJA[2], Mihaela ŠATVAR VRBANČIĆ[2], Mile MARKOSKI[3], Tomaš Lošak[4], Goran FRUK[5]

[1]University of Zagreb, Faculty of Agriculture, Department of Plant Nutrition, Svetošimunska cesta 25, Zagreb, Croatia
[2]Univesity of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia
[3]Ss. Cyril and Methodius University in Skopje, Str. 16-ta Makedonska brigada 3, Skopje, Macedonia
[4]Mendel University in Brno, Faculty of Regional Development and International Studies, třída Generála Píky 2005/7, Brno, Czech Republic
[5]University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia

Corresponding author email: tkarazija@agr.hr

Abstract

Phosphorus and iron are essential elements that are necessary for various functions of plants, but also for humans. The apple, Malus × domestica Borkh., is one of the most consumed fruits in the world, also in Croatia. Since the mentioned elements influence the growth and development of the plant and also have certain health effects on humans, the presence of these elements in apples on the Zagreb market should be investigated. After collecting and sampling the apples, the phosphorus content was determined by spectrophotometry and the iron content by atomic absorption spectrometry. Apples from retail chains statistically have the highest average phosphorus content in dry matter (0.14 % P DW), while apples from organic products stores statistically have the highest phosphorus content in fresh weight (27.29 mg P/100 g fresh weight). As for iron, the statistically highest average content in both dry matter and fresh matter was found in apples from retail chains (15.99 mg Fe/kg DM and 0.261 mg Fe/100 g fresh weight). These results could be the consequences of conventional agriculture.

Key words*: fruit quality, Malus × domestica Borkh., market distribution, minerals, nutritional value.*

INTRODUCTION

Phosphorus is a vital macronutrient essential for energy metabolism in all living organisms (Vukadinović and Vukadinović, 2011). As a biogenic element, it is crucial for normal plant growth and development, playing a fundamental role in various biochemical processes, including photosynthesis, respiration, energy storage and transport, cell division, cell enlargement, and nitrogen fixation. Additionally, phosphorus is essential for seed germination, as well as the development of roots, shoots, and flowers (Muindi, 2019). Holford (1997) emphasizes that phosphorus is the most critical nutrient, after nitrogen, limiting agricultural productivity in many regions worldwide.

Inorganic phosphorus exists in the soil in various mineral forms, bound to adsorption complexes, and is present at very low concentrations in the soil solution (Čoga and Slunjski, 2018). These low concentrations result from the rapid transformation of phosphate ions into less bioavailable forms, leading to phosphorus fixation. The availability of phosphorus is strongly influenced by soil pH and the saturation of the soil adsorption complex with bases (Čoga and Slunjski, 2018). At low pH, phosphorus forms insoluble compounds with iron and aluminum, while at near-neutral pH, it forms more soluble compounds with calcium and magnesium. At high pH, it again becomes insoluble due to the formation of calcium phosphate complexes (Muindi, 2019).

As the second most important macronutrient, phosphorus directly or indirectly regulates all biological processes within plants (Raghothama, 2005) and is crucial for growth and productivity (Malhotra et al., 2018). It significantly influences flowering and fruit

ripening (Muindi, 2019), and its deficiency can delay maturation, leading to postponed harvests and reduced fruit quality (Johnston and Steen, 2000). Furthermore, phosphorus is a key structural component of cellular membranes (Johnston and Steen, 2000) and plays a central role in adenosine triphosphate (ATP) synthesis, which serves as the primary energy carrier in biochemical processes (Muindi, 2019). Additionally, it is an essential constituent of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids, and sugar phosphates. Phosphorus is also integral to protein and vitamin biosynthesis, is present in key enzymes (Johnston and Steen, 2000), and is indispensable for the synthesis of nucleic acids and biological membranes (Lambers, 2021). Several studies underscore its critical role in photosynthesis (Johnston and Steen, 2000; Lambers, 2021; Muindi, 2019) and plant respiration (Muindi, 2019).

Iron, another essential element, is found in the soil in various soluble forms, including Fe^{2+}, Fe^{3+}, and $FeOH^{2+}$ (Vukadinović and Vukadinović, 2011; Gluhić, 2013). According to Čoga and Slunjski (2018), Fe^{2+} is moderately mobile and exists in an exchangeable form within the soil adsorption complex, whereas Fe^{3+} is more tightly bound and largely immobile.

As a micronutrient, iron is indispensable for various metabolic pathways, including chlorophyll synthesis, photosynthesis, and respiration (Ning et al., 2023). It is also involved in protein synthesis, DNA replication, and biological nitrogen fixation, highlighting its importance for plant physiological functions. Plants primarily absorb iron in the form of Fe^{2+} and Fe^{3+} ions or as chelated complexes (Vukadinović and Vukadinović, 2011). Although iron is required in smaller quantities compared to macronutrients, it plays a crucial role in fruit quality, particularly in influencing the fresh-eating attributes of produce (Nour et al., 2010).

Iron deficiency in plants manifests as chlorosis, leaf shrinkage, and stunted growth, ultimately compromising normal development and reducing fruit quality and marketable yield (Ning et al., 2023). Furthermore, iron metabolism has been closely linked to the nutritional quality of plant-based food products (Briat et al., 2007), underscoring its significance in agricultural and human nutrition.

The aim of this study was to determine the phosphorus and iron content in apples from three different sales channels in the city of Zagreb (Croatia).

MATERIALS AND METHODS

Apple fruits sampling was conducted on October 23, 2023, in the Zagreb to analyze phosphorus and iron content. Samples were collected from three different types of sales channels, with three separate locations for each channel:

- Markets (M): Savica (M1), Kvatrić (M2), and Dolac (M3)
- Retail chains (RC): Konzum (RC1), Eurospin (RC2), and Plodine (RC3)
- Organic product stores (OPS): bio & bio (OPS1), Spar Natur Pur (OPS2), and Priroda i društvo (OPS3).

Each sampling was performed in triplicate.

Label information at the sales points provided insights into the cultivation methods of the apples. Since apple samples from retail chains were not labeled as organic, they were assumed to be conventionally grown with the use of mineral fertilizers. Meanwhile, verbal inquiries with market vendors did not yield any details regarding fertilization practices. However, all samples from organic product stores were confirmed to be organically cultivated, as these stores exclusively sell certified organic products.

After collection, the average apple samples were sent to the analytical laboratory of the Department of Plant Nutrition at the Faculty of Agriculture, University of Zagreb, for chemical analysis. The fruits were peeled, sliced, and prepared for drying. They were dried at 105°C, then ground and homogenized. The samples were digested using concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave oven. Phosphorus content was determined through spectrophotometry, while iron content was analyzed using atomic absorption spectrometry (AOAC, 2023). Dry matter was measured gravimetrically by drying to a constant mass.

For statistical analysis, variance analysis (ANOVA) was applied using the SAS System

for Windows, version 9.1 (SAS Institute Inc.). The Tukey test (SAS, 2002-2003) was used to determine significant differences in the results.

RESULTS AND DISCUSSIONS

Table 1 shows the percentage of dry matter (% DM) in apples depending on the different distribution channels and points of sale. The apples from the Spar Natur Pur organic store (OPS2) have the statistically highest percentage of dry matter at 25.26%, while the apples from the Konzum retail chain (RC1) have the statistically lowest percentage at 15.52%. Table 1 also shows the percentage of dry matter (% DM) in apples sold through three different sales channels. This shows that apples from organic products stores (OPS) statistically have the highest dry matter content at 21.63%, apples from markets have a lower content of 17.11%, while apples from retail chains have the statistically lowest dry matter content at 16.52%.

Table 1. Dry matter (% DM) content determined in apple samples collected from, markets, retail chains and organic products stores

Sales channels		% dry matter			
Markets (M)	M1	15.69	e	17.11	b
	M2	17.31	d		
	M3	18.34	cd		
Retail chains (RC)	RC1	15.52	e	16.52	b
	RC2	18.24	c		
	RC3	15.79	e		
Organic products stores (OPS)	OPS1	18.40	c	21.63	a
	OPS2	25.26	a		
	OPS3	21.22	b		

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Table 2 presents the phosphorus content in the dry matter of apples across different sales channels. The highest statistically significant phosphorus concentration was observed in apples from the Plodine retail chain (RC3), measuring 0.16% P. Conversely, the lowest statistically significant phosphorus concentration, recorded at 0.11% P, was found in apples from the Kvatrić market (M2). On average, apples obtained from retail chains exhibited the highest phosphorus content at 0.14 % P, while the lowest average phosphorus concentration was detected in apples from markets, at 0.12 % P (Table 2).

Table 2. Phosphorus content in dry matter of apple determined in samples collected from, markets, retail chains and organic products stores

Sales channels		% P in dry matter			
Markets (M)	M1	0.12	de	0.12	c
	M2	0.11	e		
	M3	0.14	b		
Retail chains (RC)	RC1	0.14	bc	0.14	a
	RC2	0.13	bc		
	RC3	0.16	a		
Organic products stores (OPS)	OPS1	0.14	b	0.13	b
	OPS2	0.13	cd		
	OPS3	0.12	cd		

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Table 3 illustrates the phosphorus content (mg P per 100 g in fresh apple matter) across different sales channels. The highest statistically significant phosphorus concentration was recorded in apples from the Spar Natur Pur organic products store (OPS2), measuring 32.09 mg P/100 g in fresh matter. In contrast, the lowest statistically significant phosphorus concentration was found in apples from the Savica market (M1), with a value of 18.19 mg P/100 g in fresh matter. On average (table 3), organic products stores exhibited the highest phosphorus content at 27.97 mg P/ 100 g in fresh matter, whereas markets had the lowest average phosphorus concentration at 21.21 mg P/100 g in fresh matter.

Table 3. Phosphorus content in fresh matter of apple determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg P/100 g in fresh matter			
Markets (M)	M1	18.19	f	21.12	c
	M2	19.21	f		
	M3	25.95	bc		
Retail chains (RC)	RC1	21.01	e	23.05	b
	RC2	23.69	d		
	RC3	24.46	cd		
Organic products stores (OPS)	OPS1	25.46	bc	27.97	a
	OPS2	32.09	a		
	OPS3	26.36	b		

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

The analysis of iron content in the dry matter of apples revealed a range of 10.52 to 22.87 mg Fe/kg in dry matter (Table 4). The highest statistically significant iron concentration per kilogram of dry matter was recorded in apples from the Plodine retail chain (RC3), whereas the lowest concentration was detected in apples from the Spar Natur Pur organic products store (OPS2). Notably, the three highest iron concentrations were distributed across all three sales channels, with the Plodine retail chain exhibiting the highest value at 22.87 mg Fe/kg in dry matter, followed by the bio&bio organic products store (OPS1) with 15.44 mg Fe/kg in dry matter, and the Savica market (M1) with 15.40 mg Fe/kg in dry matter. On average, the organic products stores exhibited the lowest iron content, with a mean value of 12.17 mg Fe/kg in dry matter, while the retail chains had the highest average iron concentration at 15.99 mg Fe/kg in dry matter (Table 4).

Table 4. Iron content in dry matter of apple determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Fe/kg in dry matter			
Markets (M)	M1	15.40	b	14.11	b
	M2	12.30	de		
	M3	14.63	bc		
Retail chains (RC)	RC1	13.60	cd	15.99	a
	RC2	11.50	ef		
	RC3	22.87	a		
Organic products stores (OPS)	OPS1	15.44	b	12.17	c
	OPS2	10.52	f		
	OPS3	10.55	f		

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

The iron content in fresh apple matter was found to range from 0.211 to 0.361 mg Fe/ 100 g in fresh matter (Table 5). The highest statistically significant iron concentration was observed in apples from the Plodine retail chain (RC3), whereas the lowest statistically significant concentration, measuring 0.211 mg Fe/100 g in fresh matter, was recorded at three different locations: Kvatrić market (T2), the Konzum retail chain (RC1), and the Eurospin retail chain (RC2). On average, apples from retail chains exhibited the highest iron content at 0.261 mg Fe/100 g in fresh matter, while those from markets had the lowest average iron concentration at 0.24 mg Fe/100 g in fresh matter (Table 5).

Table 5. Iron content in fresh matter of apple determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Fe/100 g in fresh matter			
Markets (M)	M1	0.241	c	0.240	c
	M2	0.212	d		
	M3	0.268	b		
Retail chains (RC)	RC1	0.211	d	0.261	a
	RC2	0.210	d		
	RC3	0.361	a		
Organic products stores (OPS)	OPS1	0.284	b	0.258	b
	OPS2	0.266	b		
	OPS3	0.224	cd		

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

A study conducted in Romania by Campeanu et al. (2009) reported that the phosphorus content in apple dry matter ranged between 0.351 and 0.526% P in dry matter, which is higher than the values obtained in this study in Croatia, where phosphorus levels ranged from 0.11 to 0.16% P in dry matter. Conversely, findings from Sachini et al. (2020) indicated that the phosphorus content in fresh apple pulp in Brazil varied between 15.58 and 24.26 mg P/100 g in fresh matter, whereas in Croatia, the highest recorded phosphorus concentration in organic products stores was 32.09 mg P/100 g in fresh matter.

Significant variations were also observed in iron content across different studies. Gogoasa et al. (2015) reported that domestic apple varieties in Romania contained iron levels between 3.84 and 6.02 mg Fe/kg in dry matter, whereas in this study, iron concentrations were significantly higher, ranging from 10.52 to 22.87 mg Fe/kg in dry matter. The lower values observed in Romania may be attributed to the fact that the apples analyzed originated from regions with minimal anthropogenic influence. A study conducted in Italy on the 'Golden Delicious' variety also examined in the present study found an iron content of 10.25 mg Fe/kg in dry matter, which is slightly lower than the values recorded in Croatia, where the range was 11.50 to 22.87 mg Fe/kg in dry matter.

Furthermore, a study by Veo et al. (2022) analyzed the iron content in twelve apple varieties from retail chains in the Bismarck region, reporting values between 0.11 and 0.15 mg Fe/100 g in fresh matter. This is slightly lower than the iron content measured in retail chain apples in the present study, where values ranged from 0.210 to 0.361 mg Fe/100 g in fresh matter. Among the varieties collected in Bismarck, the 'Gala' variety exhibited the lowest iron content at 0.11 mg Fe/100 g in fresh matter. In the present study, iron content for the same variety was measured at 0.266 and 0.224 mg Fe/100 g in fresh matter, aligning more closely with the results of the Bismarck study, despite the samples here being collected from organic products stores.

The comparison of phosphorus and iron content across various studies suggests that multiple factors, including apple variety, growing region, cultivation practices, and analytical methods, can influence the final results.

Regarding dietary intake, the recommended daily phosphorus intake for adults is approximately 1000 mg P/day (Goyal and Jialal, 2019). The highest contribution to daily phosphorus intake was found in apples from the organic products stores, accounting for 2.80% of the recommended daily phosphorus requirement.

For iron, the daily requirement is 10 mg Fe for males over 10 years and females over 50 years, while women aged 11-50 require 15-20 mg Fe/day (Hui, 2006). Apples collected from the retail chains contributed the highest to daily iron intake, providing approximately 2.61% of the recommended daily intake for men and 1.74% for women.

CONCLUSIONS

It was found that apples from retail chains often have a higher concentration of phosphorus than apples from local markets, which can be linked to the more intensive use of phosphate fertilizers in conventional agriculture. The range of phosphorus content in the dry matter ranged from 0.11 to 0.16% P in dry matter and from 18.19 to 32.09 mg P/100 g in fresh matter at the retail chains. In contrast, there were also fluctuations in the iron content, with apples from retail chains having a higher iron content than those from organic products stores and local markets. The values determined in the dry matter were between 10.52 and 22.87 mg Fe/kg in dry matter and in the fresh matter between 0.211 and 0.361 mg Fe/100 g in fresh matter.

The results of this study contribute to a better understanding of how different cultivation and distribution factors can influence the content of important nutrients in apples.

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MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF FRESH FRUITS OF THE GERMAN PLUM CULTIVARS, TOP SERIES IN THE CONDITIONS OF THE TROYAN, MOUNTAINOUS REGION (BG)

Georgi POPSKI, Boryana STEFANOVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, 5600 Troyan, Bulgaria

Corresponding author email: g.popski@abv.bg

Abstract

The cultivars from the Top series of the breeding program Dr. Jacob, Geisenheim, Deutschland are now widespread in almost all European countries. They are of interest for modern, sustainable plum production. The data we present are from the Troyan region, an experimental field of the RIMSA. The plantation was established in 2008, with the varieties Topgigant Plus, Topking, Top 2000, Topfive, Top, Topper, etc. The biometric indicators and biochemical composition of the fruits, as well as the color parameters of the fruit skin and fruit flesh in 2021 and 2024 were studied. It has been established that the mid-ripening varieties Topgigant Plus, Topfive, Tophit have larger fruits (40-50 g). Late-ripening varieties are characterized by a very high content of dry matter and total sugars and a low content of organic acids. This makes them extremely suitable for processing, given the small mass of the fruit (Topking, Topper). The color of the fruit skin in all varieties is dark blue, with shades of purple and dark purple. The fruit flesh is yellow, juicy, sweet.

Key words*: plum, cultivars, morphology, biochemical properties.*

INTRODUCTION

Plum is a traditional cultivar for growing in the mountain and foothill regions of Bulgaria. In these conditions, the cultivars reveal different degrees of resistance to abiotic factors and diseases, which is essential when selecting suitable cultivars for growing.

For 25 years, plum selection at Geisenheim has focused primarily on improving cultivars for ripening period - early, medium and late, resistance to plum pox, sufficient resistance to major pests and diseases, and general resistance to climatic conditions (Jacob, 2007). The cultivars Topfive, Topking, Toptaste, Topper, Top and Top 2000 are the subject of the study. The four cultivars Topgigant Plus, Topstar Plus, Tophit Plus and Topend Plus represent the large-fruited market segment (>50 g).

Three of the cultivars were registered as early as 1996, showing efficiency and stable yield (Jacob, 1998).

Top, as a hybrid of Auerbacher x Stanley, is very late ripening, Top goes into fruit early and bears profusely, self-fertile, compatible with all rootstocks and tolerant of some pests and diseases. Fruits are dark blue with good culinary and processing quality for cooler climates. Extends plum harvest season by four weeks.

Topper, as a hybrid of Cacanska najbolja x Auerbacher, is medium to late ripening, produces very early and profusely, is self-fertile, has good cold hardiness and is compatible with all rootstocks, low growing. The fruits are blue, with excellent culinary and processing qualities for warmer climates. Topper is a high yielding cultivar, showing no alternability.

Tophit, as a hybrid of Cacanska najbolja x President, is late ripening. It produces early and profusely, is self-fertile, compatible with all rootstocks and tolerant of powdery mildew. The fruits are very large, dark blue, with excellent culinary qualities for all climates.

German plum cultivars are known for their large and tasty fruit, resistance to transport and comparative stability to disease. These characteristics make them suitable for cultivation in various regions, including the Troyan mountain region. At the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) - Troyan, they have already been tested, with a focus on their adaptability to climatic conditions in the region

(Stefanova & Popski, 2020; Popski, 2023; Popski et al., 2023; Stefanova et al., 2024).

Bozhkova and Savov (2016) presents results from a four-year study in the conditions of the Plovdiv region. According to the biometric analyses, the fruit weight of Topgigant Plus and Tophit Plus was over 60 g. In 2012, the percentage of flower buds destroyed by frost was estimated, with the lowest degree of damage recorded in the cultivars Tophit Plus, Topgigant Plus, and the highest in Toptast (51%). According to the results obtained, the most suitable plum cultivars recommended for cultivation in Plovdiv region are Topgigant Plus and Tophit Plus.

In Georgia, Maghlakelidze et al. (2017) recommended for further cultivation the cultivars Tophit and President for fresh production, Empress and Stanley (fresh/dry production). Which can improve the local plum assortment because they are characterized by early ripening, high productivity and high fruit quality. Tophit fruits for their study conditions ripen on 30.09, their mass is 42 g, yields for 2014-2015 are 60-70 kg per tree.

Čmelik et al. (2007) identified the qualities of Top (2005-2007) cultivars, recommending Topfirst and Tofive for early fruit ripening, (immediately after ripening the most common cultivar Cacanska lepotica), excellent quality, easy crown formation.

Bulgarian producers are interested in new cultivars and opportunities to diversify the list of cultivars in their orchards. In response to this, since 2008 the RIMSA Troyan has been exploring the cultivars of the Top series. This study presents results for the 2021 and 2024 fruit harvests and evaluates the biometric and biochemical characteristics of the fruit.

The objective of the present study is to present a comparative characterization of German plum cultivars on morphological, biochemical characteristics and fruit color parameters, under the agroecological conditions of Troyan, Mountainous region.

MATERIALS AND METHODS

The study was conducted in 2021 and 2024 in an experimental plantation of RIMSA Troyan (42°53′N 24°43′E), the altitude was 420 m. The climate of the area is favourable for the cultivation of fruit species (including plum). The average annual temperature over a 30-year base period (1988-2017) is 10.6°C; the total annual rainfall recorded is 778 mm. Early autumn frosts usually occur in late October and around mid-April at the latest.

Specifically for the study period, the factors are presented in Figure 1.

Average temperatures in 2021 are higher than those of the 20-year base period, especially in June, July, and August. In July, the temperature is 22.7°C, which is higher than the 20.7°C average for that month. This has determined the warmer summer months in recent years related to global warming, but also drought compromising fruit production in 2021. In 2024 we also observed increased temperatures compared to the base period, especially temperatures in February and March, with a significant deviation from normal rates, in February the temperature was 2.1°C compared to -0.3°C in the 2001-2020 average, provoking an earlier onset of the growing season, and a resulting delay in flowering. Summer temperatures in June and July are similar to those of 2021, with maxima of 22.2°C and 23.5°C.

Precipitation in January 2021 was significantly above average (82.8 mm compared to the long-term (2001-2020) monthly average (45.2 mm), but much less than normal in July and September (12.4 mm in July compared to 109.1 mm in the base period). In 2024, the results are more balanced by month and yet January and February rainfall are lower than the 2001-2020 average and typically the winter months are wetter. In summer, the months of June and July show relatively high rainfall rates, largely favoring higher plum yields for the 2024 crop.

In both 2021 and 2024, there are significant deviations compared to the long-term period, especially in terms of precipitation. Precipitation in July and September in 2021 is significantly less, while in 2024 the winter months are drier, with the exception of December. Temperatures, during the summer months, have shown a stable trend of increase in recent years, a typical indicator of global warming, and these trends have serious impacts on fruit production.

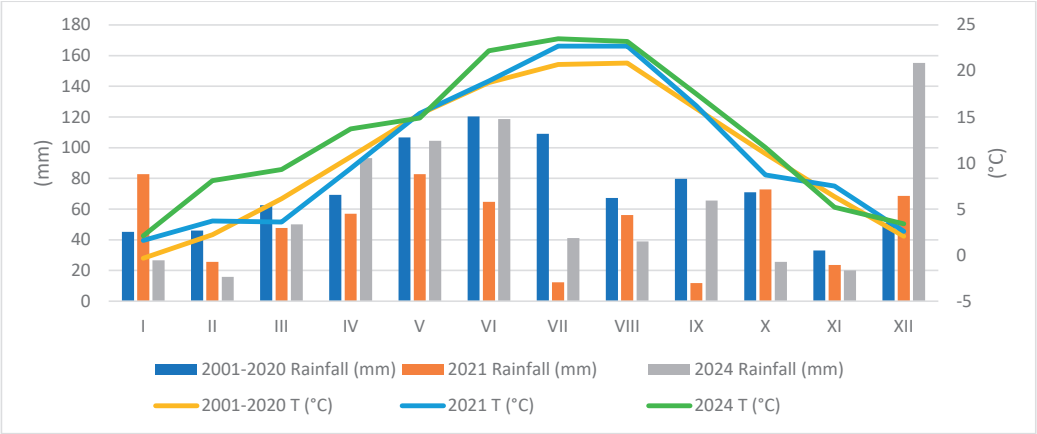


Figure 1. Average monthly temperatures (°C) and monthly precipitation sums (mm)

The German plum cultivars studied were Topgigant Plus; Topfive; Thorstar plus; p 33-6-94; Top 2000; Tophit Plus; Top; Topking; Topper. The plantation is in full fruiting.

The trees were grafted on yellow cherry plum (*Prunus cerasifera* Ehrh.) rootstock, on a 5 x 4 m scheme, grown on light grey forest soil, under non-irrigated conditions, with grassed inter-rows.

Measuring indicators

- Reproductive - yield (kg/tree), fruit weight (g), fruit size (mm) and stalk (mm);
- Chemical composition of fruit at ripening stage;
- Dry matter in refractometrically (Total Soluble Solids) (%);
- Sugars (%) (total, invert and sucrose) - according to Schoorl and Regenbogen method, (Donchev et al., 2001);
- Organic acids (malic, citric) (%) - by titration with 0.1N NaOH (Donchev et al., 2001);
- vitamin C (mg/%) to Tilman method (Donchev et al., 2000);
- Tanning substances (%) according to the methodof Levental (Donchev et al., 2000);
- Anthocyanins (mg/%) according to the method of Fuleki et al. (1968);
- Total polyphenols (mg/g) -according to Singleton & Rossi (1965);

Fruits were determined at the laboratory of RIMSA Troyan;

- color (SC-30, Colorimeter for color difference) by the CIE Lab method. Three types of colour coordinates were used during the measurement process: L, a and b;

L - colour brightness (L = 0 - black, L = 100 - white);

a - the positive values of the indicator display the amount of red, while the negative values signify the intensity of green;

b - the positive values are determinant of the yellow hues, while the negative values indicate blue.

The value of the colour tone or the dominant wavelength is represented by the a/b ratio.

The value of the colour tone or the dominant wavelength is represented by the **a/b** ratio

H (hue angle) = **b/a**. In the evaluation of H we used the most widely accepted international criterion of assigning the angle.

Statistical data processing was performed with ANOVA (Excel 2019). Treatments were declared different at p = 0.05 level of significance. The analyses were performed in three replications and the obtained values were expressed as the means ± standard error.

RESULTS AND DISCUSSIONS

In 2021, the largest fruit and stone mass were recorded in the Topgigant Plus cultivar (45.17 g and 2.00 g), while the smallest values were recorded in Top 2000 (fruit mass 16.93 g, stone mass 0.80). The relative proportion of stone for 2021 ranges between 3.69% (p 33-6-94) and 6.33% (Topper) (Table 1).

In 2024, the fruit weight ranges from 54.38 g in the Topgigant Plus cultivar to 21.62 g in the

Topper cultivar. The cultivars with higher mass are Topfive (51.78 g) and Tophit Plus (42.66 g), and it is noticeable that the mass of most cultivars has increased compared to 2021. According to Suranyi (2019), Tophit Plus reaches 50-60 g. Topper agrees with our data (28-32 g), while for Topfive it gives only 31-35 g. Of course this depends entirely on the growing conditions and climatic factors at the time of the research, as is the case in our study.
The stone mass in 2024 is 0.92 g (Top 2000), 1.14 g (Top), 1.18 g (Topking) and the highest stone mass is Topfive (2.18 g). However, the relative share of stone is highest in Topper cultivar 6.03% (small fruit mass (28.54 g), large stone mass 1.72 g), followed by 5.25% in Topking and Topper. The smallest share is in Topgiant Plus and Top 2000 (3.88%). Kovac et al. (2013), considered that Tophit has 3% and its size is 43-44 mm (Table 1).
Fruit size in 2021 was smallest in Top 2000 (height 35.30 mm, width 29.80 mm) and thickness (28.20 mm) and the largest fruit height was measured in Topgigant Plus (49.20 mm), Tophit cultivar had the widest fruit (40.60 mm), Topgigant Plus and Topfive had the largest thickness (Table 1).

Table 1. Fruit and stone morphological parameters of different cultivars of the Top series (2021-2024)

				Fruit size (mm)			*Stone size (mm)*			Fruit stalk length (mm)
	Fruit weight (g)	Stone weight (g)	Share of stone (%)	Lenght	Width	Thickness	Lenght	Width	Thickness	
					2021					
Topgigant Plus	45.17	2.00	4.44	49.20	40.20	39.80	28.20	17.20	10.00	
Topfive	38.14	1.69	4.44	45.40	39.20	37.80	29.00	16.80	10.20	
Topstar Plus	27.81	1.25	4.50	43.50	34.60	34.30	26.70	15.90	9.20	
p 33-6-94	35.56	1.31	3.69	45.00	38.50	37.30	25.40	16.80	8.40	
Top 2000	16.93	0.80	4.74	35.30	29.80	28.20	21.10	12.30	7.80	
Tophit Plus	36.56	1.55	4.24	46.10	40.60	36.20	25.89	15.67	9.67	
Top	21.51	0.95	4.42	38.10	32.90	30.20	21.20	13.90	8.50	
Topking	20.37	1.12	5.48	36.60	30.50	30.70	21.80	14.50	8.70	
Topper	21.44	1.36	6.33	39.00	32.00	30.10	25.10	14.40	8.80	
cv (%)	*33.71*	*27.79*	*16.41*	*11.59*	*12.16*	*12.27*	*11.86*	*10.61*	*8.84*	
LSD $_{0,05}$	*3.94*	*0.18*		*2.17*	*1.92*	*1.63*	*1.20*	*0.73*	*0.51*	
					2024					
Topgigant Plus	54.38	2.11	3.88	50.50	42.70	42.60	28.10	17.00	10.50	12.50
Topfive	51.78	2.18	4.21	51.00	43.00	42.90	29.10	17.90	10.90	12.29
Topstar Plus	26.30	1.19	4.51	39.50	33.00	34.40	23.70	15.00	9.40	19.29
p 33-6-94	39.61	1.76	4.44	45.10	39.10	39.40	24.33	16.89	9.33	13.50
Top 2000	23.67	0.92	3.88	37.80	33.00	33.80	20.20	13.20	8.60	15.22
Tophit Plus	42.66	1.85	4.34	46.90	40.00	40.20	24.78	15.67	10.22	15.13
Top	21.61	1.14	5.25	37.20	31.70	31.70	20.90	14.10	8.90	15.63
Topking	22.37	1.18	5.25	36.40	31.90	32.20	21.00	14.50	8.80	14.33
Topper	28.54	1.72	6.03	42.50	34.20	35.20	26.00	15.40	9.10	15.33
cv (%)	*37.19*	*29.71*		*13.17*	*12.76*	*11.86*	*13.06*	*9.79*	*8.57*	*14.11*
LSD $_{0,05}$	*4.29*	*0.15*		*1.96*	*2.03*	*1.60*	*1.06*	*0.64*	*0.46*	*1.86*

The large-fruited cultivars have fruit sizes around 50-51mm for height (Topgigant Plus and Top 2000), and their width at the abdominal suture and thickness are also the largest (42-43 mm).
The parameters of the stones were also measured. Topfive has the greatest height 29.10 mm, followed by Topgigant Plus (28.10 mm), with a width of 17-18 mm and a thickness (10.5-10.9mm).
It is noticeable that the thickness and width of the fruit are almost similar, for the relevant cultivar, with a coefficient of variation cv% of 11.86-12.76, while in the case of stone dimensions, it is noticeable that the thickness is over 8.6 mm and ranges up to 10.5-10.9 mm

(very low variation - 8.57%), while the width ranges in relation to the height in a wider range. In 2024, fruit stalk length ranged between 12.29 mm (Topfive) and 19.29 mm (Topstar plus), showing significant variation.

An increase in fruit weight and an improvement in morphological parameters is observed between 2021 and 2024. The cultivars Topgigant Plus and Topfive stand out with the best morphological performance in 2021 and in 2024. Similar results were reported by (Molnar et al., 2016). The cultivars with a lower relative share of stone and better fruit sizes deserve special attention.

All biometric indicators have very strong correlations with each other (Table 2). Fruit weight and stone weight were strongly correlated (r = 0.929686), i.e., as fruit weight increased, stone weight increased.

The extremely strong correlation between fruit weight and fruit width (r = 0.992923) indicates that fruit width is a reliable indicator of fruit weight. Practically, it can be used to quickly estimate weight when width measurement is more convenient. Mesa et al. (2021) also found this highest and significant correlation between fruit weight and equatorial diameter (r=0.96).

The strong positive correlation (r=0.982449) between fruit height and fruit thickness indicates that they change proportionally, i.e. fruit with specific shape are characteristic of particular cultivars.

A strong positive correlation between acidity and physical characteristics (e.g. r = 0.750 with fruit weight) indicates that larger fruit may contain more acid, which influences taste.

Dry matter was negatively correlated with fruit weight (r = -0.703), width (r = -0.704) and height (r = -0.710). This can be explained by the fact that larger fruits often contain more water and less dry matter. It is important to note that these relations may have significant effects on the processing of fruits and their use in different industries, for example for drying.

Table 2. Pearson correlation coefficients matrix for fruit morphological values

	Fruit weight (g)	Stone weight (g)	Lenght (mm)	Width (mm)	Dry matter (%)	Total sugars (%)	Acids (%)
Fruit weight (g)	1						
Stone weight (g)	0.929686	1					
Lenght (mm)	0.984124	0.96277	1				
Width (mm)	0.992923	0.924911	0.982449	1			
Dry matter (%)	-0.70276	-0.63834	-0.71032	-0.7037	1		
Total sugars (%)	-0.30866	-0.17774	-0.34718	-0.33317	0.561312	1	
Acids (%)	0.749764	0.663712	0.723644	0.756579	-0.75991	-0.12493	1

On average, for the conditions of the Troyan region, ripening starts at the end of August with Topgigant Plus (20-23.08) and ends at the end of September with Topper (18-25.09) (Figure 2.). In both years, the cultivars Top2000;Tophit Plus; Topking; Top and Topper ripened after 15.09. for Troyan conditions, on 15.09; 20.09; 28.09. Sotirov et al. (2021) for the Kyustendil area, which is located in southern Bulgaria and where the climate is warmer, the same late cultivars ripened up to 10-15 days earlier, namely Topking (10.08); Top2000 (25.08); Topper (08.09). The late cultivar Tophit Plus is given a timing of 15-18.09, which matches our results. Sotirov & Dimitrova (2019) show that the early cultivar Topgigant Plus is also ahead of the ripening date under Kyustendil conditions (10.08), compared to the Troyan area (20-23.08) (Figure 2). For central European conditions, Suranyi (2019), set the ripening date a month earlier for Topking 25.08 and ten days for Topper (10.09). The earlier cultivar, Topfive (12.08), matches the timing for Troyan.

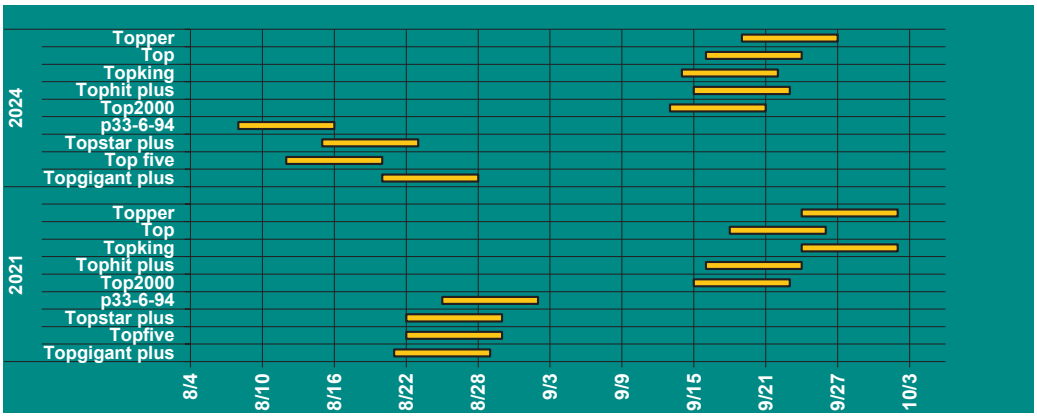


Figure 2. Ripening period 2021-2024

Yields from tree are significantly higher in 2024 compared to 2021 (Table 3). On average over the two years of the experiment, they range from 5 to 25 kg per tree. Fruit production was not regular over the years, showing alternativeness, but this depended on climatic factors and agro-technical measures applied in the plantation. The potential of the cultivars is much greater with good care.

Table 3. Yield (kg/tree) (2021; 2024)

	Topgigant Plus	Topfive	Topstar Plus	p 33-6-94	Top 2000	Tophit Plus	Top	Topking	Topper
2021	12.00	4.50	5.00	5-10	10.50	8.50	11.00	25.00	6.00
2024	35.00	28.00	10.00	2.00	15.00	45.00	18.00	25.00	12.00
Average (2021-2024)	23.5	16.3	7.5	5.0	12.8	26.8	14.5	25.0	9.0

Similar insufficient and low yields were reported by Suranyi, (2019), for the period of his research (2002-2003) 6 to 10 year old plum trees had 25-50 kg/tree yield and the fruits had low mass due to drought. It is the same for Sotirov et al. (2021), the average yields for 2019-2020 are 24 to 52 kg/tree.

The first 4 cultivars of the group are earlier ripening (end of August 18-28) than the next 5, which ripen after 15 September. This largely determines the high dry matter content in 2024 of Topper 22.5%, Top 2000 (23.5%) and Topking 27%. Topfive, Tophit Plus, Topstar Plus, and Top have dry matter contents around 21-22% (Table 4). In comparison, in 2021 the trend towards very high dry matter of this group of cultivars is confirmed (23-25.5%). We have similar data from results of Molnar (2016) Topfive (around 27%) and Kolev (2023), which also indicate a similar high percentage - Tophit Plus 22.6% Topper 22.4% dry matter for 2022 (Table 4).

For the region of Kyustendil (Kolev, 2023) found that the dry matter in fruits of the cultivars of the Top series ranged from 12.8% to 25.2%, the content of titratable acids from 0.52% to 1.47%, and of total sugars from 7.9% to 13.21%.

For the Plovdiv region, the total soluble solids content of the cultivars studied ranged from 15.7% in the Top cultivar to 24.75% in the Toptaste cultivar (Bozhkova, 2016).

In our study, total sugars for 2024 range with a cv coefficient of 22% from 13.5% (Tophit Plus) to 25.33% (Topking). In 2021 they range from 6.65% (Top 2000) to 22.00% (p 33-6-94). Accordingly, the cultivars Top 2000, Tophit Plus and Topking have the least amount of sucrose < 3% (Table 4). The variation in this parameter is the greatest, cv reaching 54%, and the values for sucrose content are between 2 and 9%, which in turn proves the absolutely suitable properties of the fruits for their inclusion in healthy menus of diabetics.

The content of titratable acids ranges from 0.47 to 0.74%, varying by a coefficient of 19.12%. Their content also determines the taste of the fruit, according to the value of the glucoacidimetric coefficient. For the 2024 conditions, the lowest value is p 33-6-94 (23.01), followed by Topgigant Plus (24,4) and Tophit Plus (25,04). The other cultivars studied have a much higher coefficient, which is due to the high sugar content or the much lower acid content, and the fruits are characterized by a very sweet taste (Topking 25% sugars, 0.47% acids) (Table 4).

Tannins range from a maximum value in Topking (0.32%), which can affect texture and tartness, to minimum values of 0.19% in Topfive and p 33-6-94.

In polyphenols, the Topper cultivar stands out with an impressive 656.03 mg/g, suggesting a high antioxidant content (Table 4).

Table 4. Biochemical composition of fresh fruit (2021 and 2024)

	Dry matter (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Acids (%)	glucoacidimetric index	vit, C (mg/%)	Tannins (%)	Anthocyanins (mg/%)	Total polyphenols (mg/g)
2021										
Topgigant Plus	15.00	16.20	16.20		0.67	24.18	10.56	0.11	13.87	192.20
Topfive	23.00	11.95	7.85	3.90	0.60	19.92	10.55	0.14	10.66	122.58
Topstar Plus	20.50	12.60	2.55	9.55	0.67	18.81	8.80	0.15	9.84	111.64
p 33-6-94	20.50	21.90	11.60	9.79	0.74	29.59	12.32	0.20	11.29	222.80
Top 2000	21.00	6.65	5.20	1.38	0.47	14.15	21.12	0.22	11.29	102.28
Tophit Plus	23.00	14.30	6.50	7.41	0.67	21.34	8.80	0.18	3.23	171.78
Top	21.50	13.80	9.40	4.18	0.67	20.60	10.56	0.20	6.45	188.28
Topking	25.50	14.30	8.70	5.32	0.67	21.34	8.80	0.13	4.03	123.96
Topper	23.50	8.70	4.50	3.99	0.60	14.50	21.12	0.22	3.87	250.75
cv (%)	18.46	39.39	58.54	56.12	15.17	27.26	41.55	23.08	49.09	29.57
2024										
Topgigant Plus	18.00	16.37	13.18	2.99	0.67	24.44	7.04	0.21	32.10	449.51
Topfive	21.00	23.23	14.08	9.15	0.74	31.39	12.32	0.19	15.48	345.25
Topstar Plus	22.00	16.38	9.86	6.52	0.60	27.30	10.56	0.21	22.90	579.84
p 33-6-94	18.00	17.03	10.76	6.28	0.74	23.01	12.32	0.19	17.90	409.41
Top 2000	23.50	14.94	12.40	2.35	0.47	31.78	10.56	0.26	28.39	517.68
Tophit Plus	21.00	13.52	11.31	2.06	0.54	25.04	10.56	0.21	21.77	275.08
Top	21.50	22.63	18.91	3.48	0.54	41.90	10.56	0.28	11.29	461.54
Topking	27.05	25.33	22.22	2.67	0.47	53.89	8.80	0.32	19.19	4.99.64
Topper	22.50	18.20	13.42	4.54	0.47	38.72	8.80	0.24	22.26	656.03
cv (%)	12.77	22.02	28.77	53.93	19.12	30.65	16.82	19.73	29.79	26.66

The colour parameters of the different cultivars were characterized by significant differences (LSD=0.05), underlining the genetic diversity. The Tophit Plus and Top 2000 cultivars stand out for their high brightness values (L) and reddish or yellow hue, which may make them preferred by consumers. As a result of different agro-meteorological conditions, there is a tendency for the parameters to change between

2021 and 2024, with an increasing trend in the values of the skin colour parameters (Table 5).
Fruit colour is an important commercial factor. Bright and intensive colours are more attractive to consumers, making cultivars with high L values and a positive 'a' (red hue) favoured. Colour parameters are key to assessing the quality of plum fruit. Differences between cultivars and variations between years underline the importance of genetic and climatic factors. Tophit Plus and Top 2000, the cultivars with bright skins and rich fruit flesh colours, have a high potential for market realisation.

Table 5. Fruit colour parameters (peel and flesh)

	Topgigant Plus	Topfive	Topstar Plus	p 33-6-94	Top 2000	Tophit Plus	Top	Topking	Topper	*LSD 0,05*
2021					fruit peel					
L	30.26	34.50	32.86	30.32	33.93	45.88	37.10	41.28	36.58	*3.20*
a	-1.62	0.57	6.07	2.79	4.69	17.47	4.95	11.54	7.88	*2.84*
b	-2.11	-4.72	-3.21	-0.19	-4.20	-9.95	-4.21	-7.94	-3.56	*2.06*
					fruit flesh					
L	41.49	39.48	44.76	48.72	47.99	44.78	42.67	43.17	47.16	*4.02*
a	-8.34	-4.70	2.25	1.78	-2.15	-5.89	-0.41	-6.16	-1.14	*1.50*
b	25.99	44.55	40.23	39.67	52.56	42.05	40.48	40.88	50.63	*8.80*
2024					fruit peel					
L	33.75	31.85	34.58	32.37	38.46	46.87	39.25	39.64	43.43	*2.82*
a	6.64	2.73	5.02	2.32	13.17	17.53	10.73	10.16	11.67	*3.13*
b	-4.95	-2.61	-4.41	-1.48	-9.38	-10.40	-7.35	-6.85	-10.25	*3.31*
					fruit flesh					
L	40.80	44.12	44.65	45.30	49.89	44.95	45.71	41.81	48.42	*3.46*
a	-5.56	-3.67	2.16	1.39	-1.07	-6.17	-2.03	-3.81	-0.15	*1.59*
b	30.58	30.75	50.67	39.15	49.63	42.14	45.56	34.86	49.77	*6.63*

Fruit flesh. The moderate positive relationship between L and a (r = 0.573814) indicates that brighter fruit flesh is redder. In 2024 the correlation between brightness and reddish hue weakens (r = 0.491082) compared to 2021
The correlation of L and b (r = 0.474621) for 2021 is a moderate positive correlation, i.e. brighter samples have a stronger yellow component. In 2024 L and b (r = 0.781454) correlate more strongly, suggesting that the yellow component becomes more important for flesh brightness (Table 6).
The a and b (r = 0.378702) are weakly positively correlated in 2021, suggesting that the reddish and yellow components are related. For 2024, a and b (r = 0.628057) are in a moderate positive relationship, stronger than in 2021
Gadze et al. (2011) considered that fruit peel colour is not a relevant indicator of fruit ripening and without analysis of organoleptic characteristics, soluble dry matter and acid content cannot be reliably used to determine harvest date.

Table 6. Pearson correlation coefficients matrix for CIELAB values and the considered colour indices of skin and fruit flesh in the cultivars of the Top series

	Fruit peel				
2021	*L*	*a*	*b*	*a/b*	*b/a*
L	1				
a	0.912973	1			
b	-0.95036	-0.82321	1		
a/b	0.299459	0.061231	-0.47156	1	
b/a	-0.02865	0.21044	0.140707	-0.1983	1

2024	*L*	*a*	*b*	*a/b*	*b/a*
L	1				
a	0.935777	1			
b	-0.91991	-0.95511	1		
a/b	-0.40932	-0.47761	0.242252	1	
b/a	0.384825	0.472973	-0.25199	-0.99179	1
	Fruit flesh				
2021	*L*	*a*	*b*	*a/b*	*b/a*
L	1				
a	0.573814	1			
b	0.474621	0.378702	1		
a/b	0.56773	0.956967	0.579242	1	
b/a	0.149051	-0.04237	-0.25152	-0.08094	1
2024	*L*	*a*	*b*	*a/b*	*b/a*
L	1				
a	0.491082	1			
b	0.781454	0.628057	1		
a/b	0.60075	0.979761	0.725407	1	
b/a	-0.51062	-0.16219	-0.40177	-0.195	1

CONCLUSIONS

We confidently recommend the plum cultivars of the Top series for adding to the plum cultivar list in the Troyan region. Earlier ripening cultivars Topgigant Plus, Topfive, Torhit Plus have larger fruits (40-50 g). Late ripening cultivars are characterised by very high dry matter and total sugars and low organic acid content. The balance between acids and sugars, as well as the ratio of fruit to stone weight, can be important for processing companies.

Peel brightness (L) correlates strongly with the reddish (a) and bluish (b) components, underlining the importance of these colour parameters for the visual appeal of the fruit. In fruit flesh, the a/b ratio is a key indicator of the reddish component of the fruit flesh. Correlations between L, a and b vary between moderate and strong, but are more strongly expressed in 2024.

The combination of high sugar content and dry matter with appropriate physical characteristics can be the key to selection.

The cultivars have a high commercial value and growers can be more competitive even on foreign markets thanks to the fact that the fruit of the whole series can be harvested for almost two months. The harvesting period they cover extends from late July to late September.

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ADVANCED CHARACTERIZATION OF PLUM FRUITS (*PRUNUS DOMESTICA* L.) BY FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AND SCANNING ELECTRON MICROSCOPY (SEM): IMPACT OF APPLIED TREATMENTS ON QUALITY AT DIFFERENT PHENOLOGICAL STAGES

Mariana RUSU[1], Iuliana MOTRESCU[2], Irina-Gabriela CARA[2], Denis ȚOPA[1], Gerard JITĂREANU[1]

[1]Faculty of Agriculture, Department of Pedotechnics, "Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700489, Iaşi, Romania
[2]Research Institute for Agriculture and Environment, "Ion Ionescu de la Brad" Iaşi University of Life Sciences, 14 Mihail Sadoveanu Alley, 700789, Iaşi, Romania

Corresponding author email: gerard.jitareanu@iuls.ro

Abstract

This study investigated plum cultivar 'Centenar' (Prunus domestica L.) from an orchard located in north-eastern Romania, using high-precision techniques such as Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The research aimed to characterize the structural and chemical dynamics of the fruits during three phenological stages: early growth phase (BBCH 71), ripening phase (BBCH 74), and maturity (BBCH 89), in relation to the use of conventional methods and treatments. FTIR analysis allowed the identification of chemical composition variations, particularly in the content of polysaccharides, pectins, and phenolic compounds, illustrating the impact of treatments and growth stage on the biochemical profile. In addition, SEM allowed a detailed observation of fruit microstructure, revealing differences in cell integrity, a factor correlated with mechanical durability and post-harvest keeping capacity. This study's results provide particularly valuable insights into the interactions between the applied treatments and fruit quality, contributing to the development of optimized agricultural strategies for plum cultivation under specific pedoclimatic conditions.

Key words*: Prunus domestica L., biochemical profile, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), phenological stages, pedoclimatic conditions.*

INTRODUCTION

Prunus domestica L. (European plum) is a significant species valued for its nutritional, medicinal properties, and economic importance. *Prunus domestica* L. has a rich content of bioactive compounds, including phenolics, flavonoids, and essential minerals. Plums are the most numerous and diverse fruittree species, making them important for current and future development due to their variety, widespread distribution, and adaptability (Arion et al., 2014)

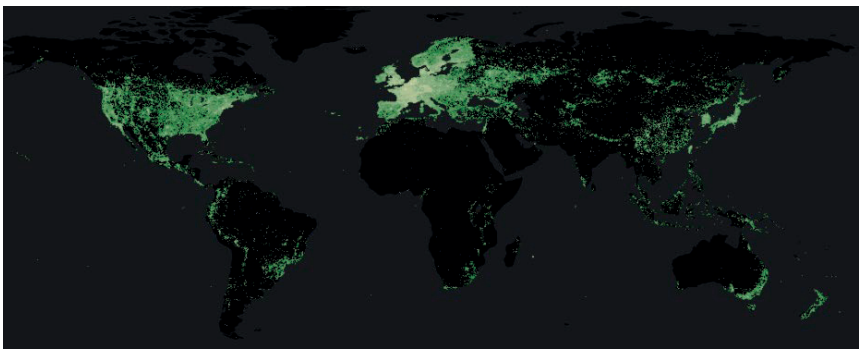


Figure 1. Distribution of *Prunus domestica* L. throughout the world (https://www.gbif.org/species/7931731)

In addition to being enjoyed as fresh fruit globally, it is also cultivated commercially consumed in dried forms and as various processed additives, finding multiple uses in the food industry (Xu et al., 2024).

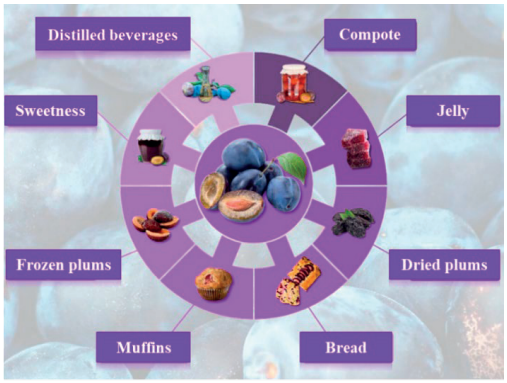


Figure 2. The versatility of plums in the food industry

Plum quality and composition are influenced by a complex interplay of factors, including soil composition, environmental conditions, and agricultural practices (Calistru et al., 2024). During phenological development, the fruit undergoes dynamic biochemical and structural changes that impact its quality and sustainability. Understanding its biochemical profile across key phenological stages - early growth phase, ripening phase, and maturity - is critical for optimizing cultivation practices. Conventional agricultural methods often employ generalized approaches that may fail to account for the dynamic biochemical and structural transformations occurring during these stages, potentially compromising fruit quality. These attributes are shaped by inherent developmental processes and specific conventional agricultural treatments, such as synthetic fertilizers, pesticides, and region-specific pedoclimatic conditions (Rakonjac et al., 2024).

In north-eastern Romania, plum cultivation is a cornerstone of local agriculture, optimizing yield requires a detailed understanding of how soil composition, environmental conditions, and agricultural practices interact across phenological stages (Cara et al., 2025). Specifically, it is critical to elucidate the biochemical and structural changes that occurduring each stage of fruit development.

This study, conducted in a north-eastern Romanian orchard, employs advanced analytical tools - Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) - to evaluate plum fruits. FTIR spectroscopy provides insights into the molecular composition of the fruit, enabling the identification of functional groups such as hydroxyl (-OH), carbonyl (C=O), and carboxyl (-COOH) associated with carbohydrates, proteins, lipids, and phenolic compounds. For instance, absorption bands around 3300 cm^{-1} and 1600 cm^{-1} correspond to O-H stretching and C=O stretching vibrations, characteristic of phenolic acids and flavonoids. Complementing FTIR, SEM provides high-resolution imaging of the fruit's microstructure, particularly the mesocarp, which determines texture, juiciness, and overall quality (Kaur et al., 2023). SEM reveals details about cell arrangement, cell wall thickness, and intercellular spaces, enabling the identification of variations in parenchyma cell morphology. For example, the presence of large, thin-walled cells with abundant intercellular spaces is associated with a soft and juicy texture. Additionally, SEM detects microstructural changes caused by ripening, storage, or mechanical damage, such as cell wall breakdown or the formation of microcracks (Basiak et al., 2019).

Traditional methods for assessing fruit quality are often time-consuming and destructive. In contrast, FTIR and SEM offer non-destructive, rapid, and reliable alternatives that provide detailed information about the elemental composition and microstructure of plum fruits (Geng et al., 2020). By integrating these techniques, this study aims to enhance our understanding of the biochemical and structural changes in plums across phenological stages, ultimately contributing to improved cultivation practices and postharvest management in north-eastern Romania.

MATERIALS AND METHODS

Research area

The research was conducted at Adamachi Farm - Iași University of Life Sciences (47°15`N, 27°30`E), located on the Moldavian Plateau in northwest Romania. The study area is characterized by a strongly anthropogenic

landscape, with an average altitude of 80-95 m. According to the Koppen climate classification, the region is characterized by a humid subtropical climate C_{fa}, with an annual average temperature of approximately 10°C and an annual rainfall of about 518 mm (https://climateknowledgeportal.worldbank.org/country/romania, accessed on 20 December 2024)

Orchard Management and Agronomic Practices

The orchard was established in 2014 using a randomized block design with three replicates. Each replicate consisted of 30 plum trees. Post-emergent, non-selective herbicides were applied to manage weed competition. Specifically, fluazifop-P-butyl (150 g/L) and isopropylamine salt (360 g a.i. L^{-1}) were administered in the spring at a rate of 2,880 mL/ha (1.39 kg a.i. ha^{-1}) to reduce competition for water and nutrients during the active growth phase of the plum trees.

Fertilization was carried out using NPK 16-16-16 fertilizer from Ameropa Company® (Targu Mures, Romania). The fertilizer was applied at a rate of 425 kg/ha, following the manufacturer's recommended doses for plum cultivation. Applications were conducted during four phenophases: BBCH 01, BBCH 60, BBCH 69, and BBCH 73, ensuring compliance with industry standards.

Collection and preparation of samples

Fruit samples of the 'Centenar' variety were collected at different growth stages - BBCH 71 and BBCH 74 - and at full maturity in August 2023 (BBCH 89). The collection process was conducted with utmost precision to ensure accuracy and reliability in the analysis. The composite samples were stored in polyethylene bags and cleaned with ultrapure water to eliminate any residual chemicals and debris. After cleaning, the samples were dried under mild conditions (25-30 degrees, protected from sunlight), and the skin and pulp were manually separated. The pulp was freeze-dried (BIOBASE BK-FD10T equipment, Jinan, China) at −42°C and 0.10 mbar for 48 hours until reaching a moisture content of 7% (MF-50 moisture analyser, A & D Company, Tokyo, Japan). The freeze-dried pulp was then ground using MC 12 equipment (Stephan, Germany), and stored in zip-lock bags covered with aluminum foil at room temperature for future use.

Research Methodology

The identification and analysis of aliphatic, phenolic, aromatic, and carbonyl functional groups through infrared spectroscopy facilitate the distinction of the chemical composition phenological stages of the plum fruit powder. For these analyses, an Interspec 200-X FTIR spectrometer was employed.

The morphology, structure, and elemental composition of the plum fruit powder were investigated using a scanning electron microscope (Quanta 450, FEI, Thermo Fisher Scientific, Hillsboro, USA) in conjunction with an energy dispersive X-ray detector (EDX) (EDAX, AMETEK Inc., Berwyn, PA, USA). Each sample was applied on a thin layer of approximately 2 mm onto aluminium stubs with the help of double-sided carbon tape, the stubs being subsequently placed within the analysis chamber. The EDX spectral analysis was conducted using EDAX Inc.'s TEAM version V4.1 system. The samples were analysed under low vacuum conditions, with a magnification of 500× (10 μm), an electron acceleration voltage of 15 kV, and a pressure of approximately 6.1×10^{-4} Pa.

RESULTS AND DISCUSSIONS

The IR spectra of plum fruits, selected at three different stages of maturation (BBCH 71, BBCH 74, BBCH 89), were obtained to analyse variations in the nature of functional groups, illustrating changes in chemical composition throughout fruit development.

The spectra obtained in different stages of maturation are illustrated in Figure 3. The IR spectra of the fruits at all three stages of development were more or less similar.

The characteristic bands observed in the 3600-4000 cm^{-1} region are associated with the vibration of hydroxyl functional groups, and the absorption was caused by the stretching of free or H-bonded OH groups (Thirumavalavan et al., 2011). This functional group (-OH stretching) is present in all stages of fruit maturity and can be attributed to the presence of water (after the lyophilisation process) or polyphenols.

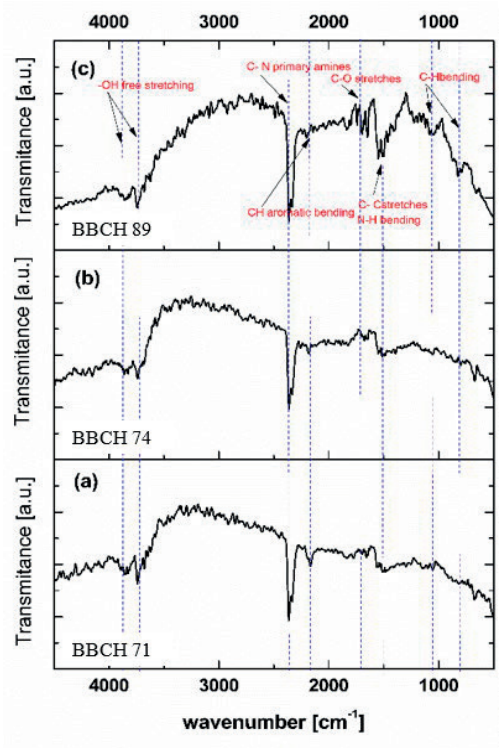


Figure 3. FT-IR spectra of plum fruit at BBCH 71 (a), BBCH 74 (b), BBCH 89 (c)

A decrease in transmittance in this region at the BBCH89 stage indicates a reduction in water content or a modification of polar compounds due to maturation processes. Characteristic C-H vibrational peaks (from aliphatic chains, such as lipids or sugars) are present at all stages, the most intense specific band being observed in BBCH 89. The relatively constant intensity suggests the stability of hydrocarbon structures, but subtle changes reflect the accumulation of sugars or fatty acid degradation.

The absorbance at ~1700 cm^{-1} (C=O vibrations) is significant, indicating the presence of carbonyl groups from organic acids, esters or ketones. An increase in this peak can be observed in BBCH89, corresponding to the accumulation of reducing sugars or volatile compounds in the baking phase.

For wave numbers below 1500 cm^{-1}, one can observe that the so-called fingerprint area of the spectra is a complex one, associated with bending and coupling vibrations and shows marked differences between stages. The modifications may be related to cellulose/pectin degradation (peaks at ~1200 cm^{-1}) or to the synthesis of phenolic compounds (peaks at ~1600 cm^{-1}).

FTIR spectra reflect the biochemical evolution of plum fruit during ripening. Stages BBCH 71 (early growth) and BBCH 74 (intermediate ripening) show compositions richer in water and polar compounds, while BBCH 89 (advanced ripening) indicates accumulation of secondary metabolites (e.g., phenolics, esters) and reduction of structural components. These changes are consistent with natural fruit transformation processes, including cell wall degradation and synthesis of compounds responsible for flavour and texture.

In addition, the intense band below 1000 cm^{-1} shows higher cellulose content during growth, observed better in BCCH 89 (Sanchez-Rivera et al., 2010).

The changes in the microstructure of the plum fruit with respect to growth and development were imaged using SEM and are illustrated in Figure 4. With the advancement of the maturity stage, the microstructure drastically changed with irregular shapes and larger intercellular areas. The appearance of the sample corresponding to the early development stage (Figure 4a) looked hazy with no particular prominent structure. With the progress of maturity (BBCH 74) some microstructures (Figure 4b) were observed. The difference in the micro-appearance is also related to the variation in chemical compositions. SEM images at stages BBCH 71 and BBCH 74 clearly revealed the microstructure of starch and carbohydrates.

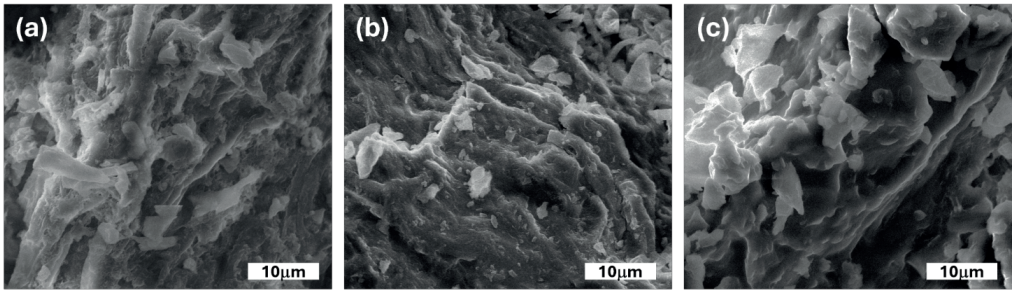


Figure 4. SEM images of plum fruit growth stages at BBCH 71 (a), BBCH 74 (b), BBCH 89 (c)

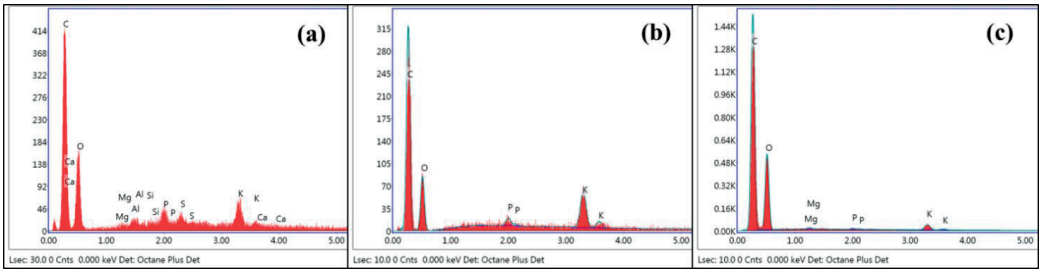


Figure 5. EDX elemental composition of plum fruit at BBCH stages 71 (a), 74 (b), and 89 (c)

EDX analysis (Figure 5) revealed that in the analysed samples, the main elements are C, O, P, and K. K was the most abundant mineral present in all stages, followed by P and Mg. EDX shows the presence of the K element in all three samples (a, b, d). In the case of sample (c), the analysis showed a decrease in the intensity of the peak corresponding to potassium. Also, for sample (a) an individualisation of the intensity of the peak corresponding to calcium was observed, with the rest of the elements identified in samples (b) and (c) being detected in negligible percentages. The composition not only varies during phenophases, but also between different varieties (Motyleva et al., 2017).

CONCLUSIONS

The present study was focused on evaluating the effect of fruit maturation on biochemical and functional compounds in the plum fruits at various stages. The results revealed that this is a potential source of many functionally important nutritional and bioactive compounds. K was the most abundant mineral present in all stages, followed by phosphorous and Mg. FT-IR analysis confirmed the presence of various functional groups and indicated the complex nature of the plum fruits. SEM clearly evinced that the microstructure of the fruits changes drastically, and the degradation of starch and other compounds occurred at the fully matured stage. The SEM images highlight the complex structure, which reflects the quality of the lyophilization process and the potential applications of the powder in the food or pharmaceutical industry (BBCH 89).

Hence, the present study favourably justifies that the plum fruits have enormous potential for commercial application as a source of nutritional and functional compounds which can add a higher value to this locally important crop.

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PRELIMINARY DATA ON THE ADAPTABILITY OF NATIVE ALMOND VARIETIES IN SOUTH-EASTERN ROMANIA

Nicoleta-Claudia SANDU, Mihaela-Cecilia DOGARU, Damian DRAGOMIR, Viorel-Cătălin OLTENACU, Mihaela OLARU, Andreea Mihaela BRASLĂ, Vlăduț HOLT

Fruit Research and Development Station Băneasa, 4 Ion Ionescu de la Brad Boulevard, District 1, 01381, Bucharest, Romania

Corresponding author email: sandu.nicoleta977@gmail.com

Abstract

This study presents preliminary data on the adaptability of two Romanian almond (Prunus amygdalus L.) cultivars, 'Mirela' and 'Veronica', under the specific eco-pedo-climatic conditions of southeastern Romania, a region characterized by heavy clay soils and increasing climatic variability. The experiment was established in December 2022 at the Moara Domnească Experimental Base, Ilfov County. The main objective was to evaluate the viability of trees during the establishment, as an indicator of adaptability to local abiotic stress factors. Soil analyses were performed before and after planting, while climatic data were collected through an automated weather station.
Results showed a survival rate of approximately 75% for both cultivars, despite the recorded precipitation deficit in 2023 (281.94 mm). The analysis of the soil revealed high clay content, slightly acidic pH in the surface horizon (6.17), and a sharp decline in available phosphorus with depth, whereas mobile potassium increased in lower horizons. The study provides a scientific basis for extending almond cultivation into non-traditional regions of Romania and highlights the importance of continued monitoring in the context of climate change.

Key words*: almond, local cultivars, adaptability, soil fertility, viability, southeastern Romania.*

INTRODUCTION

Almond (*Prunus amygdalus* L. sin., *Amygdalus communis* L.) belongs to the Rosaceae family, subfamily Prunoidae, group Amygdalae and has its origins in the mountainous regions of Central Asia (Grasselly, 1976). This species is adapted to a Mediterranean climate with hot summers and mild, dry winters (Băluță & Stănică, 2023).
The optimum temperatures of the species are between 12 and 35°C, and the absolute minimums are 10°C and the maximums are 40°C (outside the absolute temperature range, growth stops). Frost resistance is very low, the limit being -22°C (Coman et al., 2014). Cold resistance decreases as the trees approach the start of vegetation. Open flowers are destroyed at -2.0°C and set fruits do not withstand if the temperature drops below -1.5°C (Sumedrea et al., 2014). The ability to develop a deep and extensive root system allows it to better access water and nutrients. It can also grow on less fertile soil. It prefers loamy or sandy-loamy soil, which allows good aeration and water infiltration. Excess moisture increases the risk of root diseases. Also, the soil pH should be neutral to slightly alkaline. Acid soils are commonly amended with lime (calcium carbonate or similar materials) to raise the pH and improve growing conditions for most crops (Brady & Weil, 2016).
In our country, almonds have found optimal climatic conditions in the southeast, in Constanta, Valu lui Traian, Mangalia, Tulcea, in the Banat Region, the Oltenia Region and generally along the Danube until it flows into the Black Sea.
Almonds have an exceptional nutritional and medicinal importance, due to their rich content in proteins and healthy fats, vitamins and minerals. They contribute to reducing the risk of cardiovascular diseases and improving brain function (Kalita et al., 2018).
They are also widely used in the cosmetic industry, primarily due to the properties of almond oil, which is rich in vitamins E and A and is known for its moisturizing, nourishing, and anti-inflammatory effects on the skin and hair (Kaur & Saraf, 2010; Hamid & Sarmidi, 2014).
All these benefits of almonds, along with support from European funding schemes and

national agricultural development programs, have led to an increase in almond cultivation areas across Romania. This trend positions the country to become a competitive almond producer within the European market (Melhaoui et al., 2019).

The objective of this study is to evaluate the influence of soil and agro-climatic factors on the adaptability and physiological behaviour of two almond (*Prunus dulcis*) cultivars, 'Veronica' and 'Mirela', under the specific pedoclimatic conditions of the Moara Domnească area. This location presents a challenging environment for almond cultivation due to its soil composition, which has a high clay content - a characteristic generally not recommended for this species. The experimental orchard is currently in its second-year post-establishment. Given this early stage of development, the primary parameter evaluated in this investigation is the survival rate (viability) of the young trees, serving as an initial indicator of their resilience and potential for adaptation.

MATERIALS AND METHODS

The study was conducted at the Moara Domnească Experimental Base, which is part of the Research and Development Station for Fruit Growing Băneasa. The location is situated in the northeast of Bucharest, in the Vlăsia Plain (a subunit of the Romanian Plain), Ilfov County, approximately 25 km from the capital (Lat. 44.50418179, Lon. 26.24626838).

The experimental orchard was established at the end of 2022 by planting two native almond varieties - 'Veronica' and 'Mirela'. The trees were planted at a spacing of 4 meters between rows and 2 meters within rows, resulting in a planting density of approximately 1250 trees/ha. The plantation is equipped with a drip irrigation system, and standard almond cultivation practices were applied throughout.

The 'Veronica' cultivar is characterized by low to medium vigor and a slightly open canopy structure. It blooms in the middle of the season, and fruiting occurs predominantly on spur clusters and mixed shoots. The fruits are medium-sized, oval-shaped, slightly pointed at the tip, and convex on the dorsal side. The shell is greyish green in color and covered with fine hair. The kernel is oval, with a pointed tip, pearly white in color, and has an average weight of approximately 2.2 g. This variety shows good tolerance to fungal diseases and drought, which contributes to lower phytosanitary treatment costs. It is a productive cultivar, with an average yield of up to 2.3 kg of kernel/tree (Băluță, 2022).

The 'Mirela' cultivar exhibits medium vigor and has a slightly open growth habit. Its blooming period is mid-to-late and extends over a relatively long duration. Fruiting occurs mainly on spur clusters. The in-shell fruits are medium to large, with a thick shell of medium hardness when cracked. The kernel is short but broad, with an average weight of approximately 2.5 g, and a low incidence of double kernels. This variety is productive but shows some sensitivity to alternate bearing. The fruits contain low levels of mineral elements but are rich in oils, with a low linoleic acid content and a high oleic acid content, according to the fatty acid profile. The oleic acid content varies between 60% to 75%, linoleic acid content is between 16% to 30% (Yada et al., 2011). Together, these two fatty acids typically make up over 85-90% of the total lipid content in almonds (Houmy et al., 2014). The fruits of both cultivars can be consumed fresh or processed for use in the food and pharmaceutical industries (Opriță et al., 2022).

The soil type at Experimental Base Moara Domnească is a reddish preluvosoil.

To determine the suitability and degree of adaptability of almond to the pedological conditions in the study area, Moara Domneasca, soil samples were taken to analyze its main physical and chemical properties. The evaluation of these characteristics is essential for establishing the soil's capacity to support healthy almond growth, considering the species' specific requirements for texture, structure, pH and nutrient content.

The samples were analyzed according to the "Methodology for the Elaboration of Soil Studies" – I.C.P.A. Bucharest, 1987, in the Agrochemistry Laboratory of the Fruit Growing Research and Development Station Băneasa, Bucharest (Dogaru, 2024).

Soil Chemical Analysis Determinations

Soil sampling was carried out according to STAS 7184/1-84, at a depth of 0-20 cm. Each soil sample consisted of 20 subsamples,

combined to represent the average characteristics of the soil.

In the laboratory, the samples were analyzed using STAS methods, also employed by ICPA Bucharest, as follows:

- **pH** was determined in aqua extract, with a soil-to-water ratio of 1:2.5, using a Hanna pH meter, according to SR 7184-13.
- **The colorimetric determination of mobile phosphorus** in ammonium lactate-acetate extract (PAL) was carried out using the Egner-Riehm-Domingo method. The values were read using a UV-VIS spectrophotometer (CAMSPEC M 330), and the results were expressed in ppm, according to STAS 7184/19-82.
- **The determination of mobile potassium content** (KAL) was performed in ammonium lactate-acetate extract, using the Egner-Riehm method, through flame photometry. The results were expressed in ppm, in accordance with STAS 7184/19-82.
- **The humus content** was determined by wet oxidation using the Walkley-Black-Gogoaşă method, followed by titrimetric measurement, according to STAS 7184/21-82.

Depending on the soil pH, additional agrochemical indicators of fertility were also determined:

- **Hydrolytic acidity (Ah)** was determined using the Kappen method, with results expressed in me/100 g soil.
- **Sum of exchangeable bases (SB)** was determined by the Kappen method as well, and results were also expressed in me/100 g soil, according to STAS 7184/12-88.
- **Base saturation percentage (V_{Ah})** was calculated using the formula V_{Ah} = SB * 100/ (SB + Ah), according to STAS 7184/12-88.

RESULTS AND DISCUSSIONS

The following soil characteristics were determined (by granulometric analysis for determination of the soil content in clay, dust and sand): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm, to 41.63% at a depth of 41-53 cm and 47.39% at depths greater than 54 cm (Table 1).

The clay texture determines a low mobility of nutrients and a poor permeability of the water in the soil (Negrușier et al., 2024). Under these conditions, the almond root system is prone to asphyxiation and fungal infections, manifested by stunted growth and reduced plant vigor.

A study conducted in almond production areas in Spain showed that clayey soils can induce root asphyxiation problems and stress caused by flooding, significantly affecting tree survival and growth (Forcada et al., 2020).

Table 1. The granulometric composition of the soil (Experimental Base Moara Domnească, 2019)

Horizon	**Ao**	**Ao/Bt**	**Bt**	**C**
Depth(cm)	0-40	41-53	54-200	Over 200
Clay (%)	40.55	41.63	47.39	36.18
Coarse sand (%)	0.36	0.52	0.37	0.42
Fine sand (%)	34.33	21.54	27.59	32.04
Dust (%)	24.75	56.28	30.34	32.04
Texture	Clay loam	Clay loam	Clay loam	Clay loam

- **Nitrogen index (IN)** was determined as IN = H * V_{Ah}/100, in accordance with STAS 7184/12-88

Climatic condition can be an important factor in evaluating almond adaptability. Climatic data on temperature and precipitation were collected using an Adcon automated weather station, operational since its installation on June 5, 2020.

The soil content in humus is good in the first 40 cm of profile, where most of the roots of young trees are located, reaching the value of 3.26%, then suddenly decreases up to 1% in the Bt horizon profile (Table 2).

Total nitrogen is higher in the superficial layer (0.144%) and decreases in depth (0.07%), following the typical trend of organic matter mineralization. Total cation exchange capacity values (28.04-30.0 meq/100 g) indicate a high nutrient retention capacity, specific to soils with high clay and organic matter content (Dogaru & Damian, 2021).

The pH is slightly acidic at the soil surface (6.4), reaching alkaline (8.3) in the C horizon

Almonds tolerate slightly acidic pH, but do not provide ideal conditions for the efficient absorption of essential nutrients, especially calcium, magnesium and phosphorus (https://www.yara.us/crop-nutrition/almond/growth -requirements/). For optimal and healthy development, the pH should be between 6.5-8.0 (Muhammad et al., 2017).

Table 2. Physical and chemical properties of the profile soil (Experimental Base Moara Domneasca, 2019)

Horizon	Ao	Ao/Bt	Bt	C
Humus, (%)	3.26	1.87	1.00	1.00
Soluble Ca (mg/100g soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exceangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Phosphorus soluble in AL (mg/100 g soil)	50	40	40	30

Other indicators like the nitrogen index (NI), hydrolytic acidity, humus, organic carbon and so on were determined during before planting (2022).

One of the most important factors in agriculture is the environment in which the plant grows and develops. Substrates must be able to provide the necessary water, nutrients and oxygen for plants, as well as support for the whole plant (Miller & Jones, 1995).

The range of analyses, which includes pH, potentially available phosphorus content, potentially available potassium content, and humus content, highlights the soil's fertility status (Table 3).

With values of 2.70 and 4.50, the nitrogen index in the analyzed profiles (2022) indicates a good to very good nitrogen fertility, which supports a proper vegetative growth in the early stages of almond development.

Soluble phosphorus (AL) levels were adequate in the 0-20 cm soil layer (81.11 mg/kg) but decreased notably in the 20-40 cm layer (55.56 mg/kg), which may adversely affect deep root system development, particularly during the establishment phase. These values are adequate for almond cultivation (according to Romanian agrochemical norms: >30 mg/kg = well supplied). The mobile potassium content is high (192-233 mg/kg) in both layers, which favors the flowering and fruiting processes. Potassium is essential for drought resistance – an important advantage in the climatic conditions of 2023.

The degree of base saturation is over 90% in the upper horizons (very good values), which reflects a soil well supplied with essential cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}).

Fertility indicators, such as humus content, total nitrogen, and mobile potassium, exhibit progressively higher values with increasing depth of the analyzed soil profiles. This trend indicates a greater accumulation of nutrients in the lower horizons, which may be utilized by employing rootstocks with deep rooting systems. Such rootstocks can access these resources in deeper soil layers, thus contributing to the sustainable development of almond cultivation.

Table 3. Chemical properties of the soil, before planting (Experimental Base Moara Domneasca, 2022

Depth (cm)	0 - 20	20 - 40
pH	6.17	6.31
Soluble Bases (mg/100 g soil)	24.95	24,57
Hydrolytic acidity (mg/100 g soil)	1,82	2.60
Humus, (%)	2.90	4.98
Organic Carbon	1.68	2.89
Total cation exchange capacity	26.77	27.17
Degree of saturation in bases (%)	93.20	90.43
Nitrogen index	2.70	4.50
Phosphorus soluble in AL (mg/kg)	81.11	55.56
Potassium soluble in AL (mg/kg)	192.00	233.00

Another significant limiting factor for almond adaptability is climatic conditions, especially temperature and precipitation. The alternation of temperatures during winter, characterized by periods of warming followed by the sudden return of frost, negatively affects almond flower buds. This thermal variation can lead to the premature triggering of physiological processes in the buds, which thus become more sensitive to subsequent low temperatures, resulting in significant fruit losses (Keleta et al., 2022).

Moara Domneasca, located in Ilfov County, Romania, has traditionally exhibited a temperate-continental climate, marked by warm summers and cold winters. However, beginning in 2022, notable alterations in the local climatic

regime have been observed, likely attributable to broader climate change trends.

The average annual temperature in the area is approximately 12°C, and annual precipitation typically ranges between 550 and 600 mm. In this study, we considered the mean values of temperature and precipitation.

In 2023, extreme temperature variations were recorded. The absolute minimum was observed in February (-8.6°C), while the highest temperature occurred in July, reaching 40.5°C, surpassing the maximum recorded in 2022. The average monthly temperatures in 2023 ranged from a low of 3.2°C in February to a high of 26.0°C in August (Figure 1).

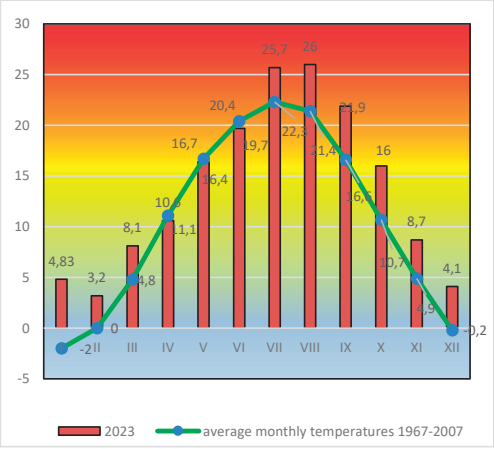


Figure 1. Annual average temperatures 2023 compared to multi-year average temperatures - Moara Domnească

Regarding precipitation, the year 2023 was exceptionally dry. Almond (*Prunus amygdalus*) is considered a species with good drought tolerance.

However, the total rainfall recorded in 2023, of only 281.94 mm, representing less than half of the multiannual average of 610.9 mm, is significantly below the average water requirements of almond, which vary between 600 and 800 mm annually (Egea et al., 2010; Goldhamer, 1999).

Precipitation distribution throughout the year was uneven (Figure 2), with the lowest recorded in September (0.3 mm) and the highest in April (59.4 mm).

This irregular distribution of rainfall affected the percentage of establishment and initial development of young almond tree.

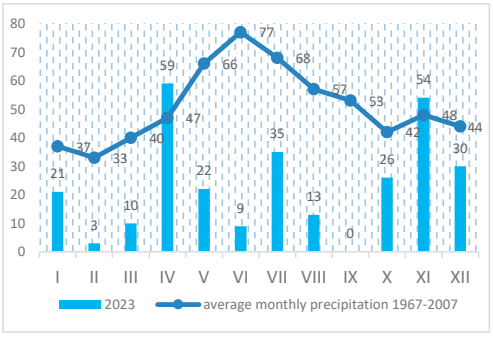


Figure 2. Average annual precipitation 2023, compared to multi-year averages (Source:own processing)

The lack of available soil moisture, particularly during the spring and summer months, critical periods for root system initiation and adaptation to the environment, can lead to losses among the planted material. These losses are reflected in the tree viability rate, calculated as the ratio between the number of dried trees and the total number of trees planted for each cultivar (Table 4). Thus, the percentage of losses was relatively similar between the two cultivars analyzed, with 24.33% for the 'Mirela' cultivar and 24.77% for 'Veronica'.

Table 4. Almond Tree viability in 2023 (Source: own processing)

Almond Cultivar	Viable Trees	Total Trees	Dry Trees (%)	Viable Trees (%)
Mirela	225	297	24.33	75.45
Veronica	113	151	24.77	75.67

This close percentage indicates that the climatic variations during the winter did not clearly favor or disadvantage either variety. It can be concluded that both cultivars demonstrated comparable adaptability to the local pedoclimatic conditions, and the observed tree losses are more likely the result of cumulative factors (such as planting stress, post-transplant shock, or localized deficiencies) rather than a direct consequence of temperature or precipitation extremes during the evaluated period.

The high temperatures recorded in 2023 did not appear to be the primary limiting factor, as tree establishment rates remained relatively high. However, the severe precipitation deficit created significant water stress for the young trees, negatively affecting their viability (Figure 3).



Figure 3. Correlation between Climatic Indicators and Almond tree Viability by cultivar (2023) (Source: own processing)

It is worth emphasizing that the implementation of an irrigation system is essential to alleviate water stress, support root system development, and ensure the overall physiological performance and resistance of almonds. In the context of prolonged periods of drought, supplementary irrigation plays an essential role in ensuring the long-term sustainability of almond crops. This aligns with the findings of García-Tejero et al., (2019), who demonstrated that deficit irrigation strategies, when properly managed, can enhance water-use efficiency without compromising yield, making them a sustainable approach in semiarid Mediterranean environments.

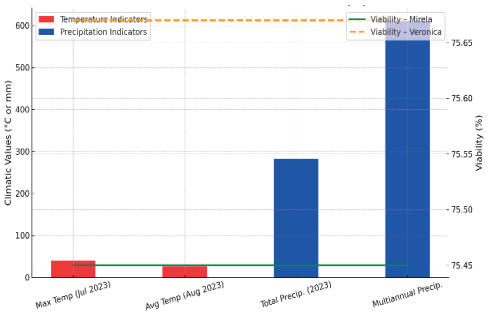


Figure 4. Image with almond tree 'Mirela' (Source: original photo)



Figure 5. Image with almond tree 'Veronica' (Source: original photo)

CONCLUSIONS

The preliminary results of the study conducted at the Moara Domneasca experimental base highlight a promising adaptability of the two Romanian almond cultivars, 'Veronica' and 'Mirela', to the pedoclimatic conditions of southeastern Romania. These findings suggest that almond can be successfully cultivated in the Moara Domneasca area, provided that appropriate technological measures are implemented, such as:

- selection of rootstocks suited to heavy-textured soils.
- application of soil amendments (organic and mineral) to improve soil structure.
- implementation of effective drainage systems and soil loosening operations.
- differentiated fertilization, considering the uneven distribution of macronutrients.
- use of leguminous intercrops to help loosen the soil.
- application of mulch (straw, leaves) for soil protection and enhancement.

The correlation between climatic data and the planting success rate highlighted the crucial importance of drip irrigation during the critical rooting months (May-August).

The results highlight that young trees establishment and early development are influenced not only by climatic conditions but also by a combination of pedoclimatic and technological factors, including planting stress and soil-specific fertility.

In summary, the cultivars 'Mirela' and 'Veronica' demonstrate stable agronomic performance under stress conditions,

representing a viable option for the expansion of almond cultivation in Romania.

However, it is essential to continue medium- and long-term research to evaluate fruiting performance, kernel quality, and disease dynamics in the context of current climate change.

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ORGANIC FOLIAR FERTILISERS INFLUENCE ON SOME PEACH VARIETIES

Alina STOICHESCU, Ionuț DASCĂLU, Tamara Edina GAL, Daniela Nicoleta SCEDEI, Olimpia Alina IORDĂNESCU

University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, 300645, Timişoara, Romania

Corresponding author email: ionut.dascalu@usvt.ro

Abstract

Foliar fertilization is a fundamental instrument in orchard management, having an important role in obtaining good-quality crops and being essential in maintaining fruit yield, as frequent fertilization assures sufficient mineral nutrients for tree growth. Foliar fertilisers have countless benefits for plants, produce a rapid effect in comparison with soil fertilization and are easily assimilated. Foliar fertilisers also increase plant resistance to diseases and pests thus decreasing the environmental impact of chemical fertilisers. The presented data are partial results of the PhD thesis. The experiment was conducted in 2024 at a fruit tree nursery located in Lugoj, Timis County (45°42'22.1"N 21°51'36.1"E). The research focused on analyzing four peach varieties: "Tokinostate", "Eureka," "Desert Gold" and "Elbertina." These varieties were treated with three different organic foliar fertilisers - Albit, Cropmax, and Naturamin Plus - applied during three distinct growth phenophases. The studied varieties were differently influenced by the applied fertilisers. Soluble solid content was improved in most of the varieties when using organic fertilisers.

Key words*: fruit mass, soluble solid content, size index, fruit hardness.*

INTRODUCTION

Fertilization of fruit trees is an essential component of technology since it has a substantial impact on crop quality and production (Baldi et al., 2005, Dascălu et al., 2023). Climatic conditions and management techniques have a substantial effect on the efficiency of fertilization (Cai et al., 2023). Organic fertilisers can improve peach root production and lifetime (Baldi et al., 2010).

Foliar fertilization has become an essential management technique in intensive orchards offering a promising potential for improving fruit quality at a low cost and low environmental effect (Csihon et al., 2021). Sprays deliver nutrients to plants faster than soil fertilization (Septar et al., 2022). The quick responsiveness, efficacy and eradication of deficiencies caused by nutrients make foliar spraying superior to soil treatment (Ali et al., 2014). When conducting foliar spray experiments, physico-chemical characteristics must be taken into consideration (Fernández et al., 2006). Foliar fertilisers, especialy the organic ones, can have significant positive influences on the soluble solid content of the fruits (Dascalu et al., 2024).

In terms of both economic and nutritional value, peaches (*Persica vulgaris* L.) rank as the second most significant deciduous fruit crop in the world (Cai et al., 2023). Peaches contain antioxidant phytochemicals, vitamins, minerals, fibres, which have beneficial effects on human health (Tomás-Barberán et al., 2013, Byrne et al 2012). This species responds well to fertilization and is a major consumer of N and K (Damianov et al., 2022).

For hundreds of years, peaches have been a staple of the human diet. In the last decades, however, the amount of peaches consumed per person has decreased significantly (Bento et al., 2022; Anthony & Minas, 2021).

According to consumer surveys, fruit that is unpleasant, browned, not at optimal maturity (such as overripe or too green), or has an unusual texture are the reasons why peach consumption has decreased (Crisosto & Labavitch, 2002; Koneru, 2013).

Fertilization is a preharvest factor with great influence on fruit quality (Minas et al., 2018). For example, an excessive fertilization with N prevented peaches from accumulating sugar (Jia et al., 1999), possibly because of the shading of

the fruits by the surrounding rich foliage (Wert et al., 2009).

MATERIALS AND METHODS

Four peach varieties with a close ripening period (second decade of August) were analyzed for the research: "Tokinostate", "Eureka", "Desert Gold" and "Elbertina" (Figure 1). Each variety was treated with 3 organic foliar fertilisers at three different growth phenophases, and for each variety controls were chosen which were not fertilized. The used foliar fertilisers were: Albit (dose: 150 mL/ha), Cropmax (dose: 1.5 L/ha), Naturamin Plus (dose: 1 kg/ha). The first treatment was applied in the second decade of May and the following treatments were applied about 21 days apart.

The experiment took place during 2024, at the nursery of the University of Life Sciences "King Mihai I" from Timişoara, in Lugoj, Timis County (45°42'22.1"N 21°51'36.1"E).

The trees on which the experiment was conducted were planted in 2015, at distances of 4x4 m. Oradea rootstock was used when the four peach varieties were grafted, and their crown shape has been formed to be an "improved vase".

In order to determine the influence of fertilisers, the following parameters were analyzed on the fruits: size index, mass, hardness, soluble solid content and stone percentage. For each parameter, 15 fruits picked at optimal maturity were analyzed. The estimated yield per tree was also determined.

Determination of the size index resulted from caliper measurements on the large diameter, small diameter and height of the fruit, and then the SI=(D+d+h)/3 (SI=Size index; D=large diameter; d=small diameter, h=height) formula was used.

The mass of the fruit was measured using the Kern PES620-3M analytical balance. Fruit hardness was determined with the Lutron FR-5105 penetrometer and the soluble solid content was determined with the ATAGO PAL 3870 digital refractometer. The stones were weighed separately and the stone percentage was determined in relation to the fruit weight.

Concerning the fruit yield per tree, about 10 days after the last spraying, fruits of each tree were counted, and after harvesting, the average mass of 30 fruits was calculated and multiplied by the number of fruits.

Data were analyzed using SAS Studio SAS® Studio 3.8 software's One Way Anova test.



Figure 1. The studied varieties and genotypes of peaches

RESULTS AND DISCUSSIONS

The results determined for the examined parameters are presented in Figures 2-7.

Size index

Size of the fruits of "Tokinostate" variety ranged from 52.09 mm (Control) to 61.05 mm (Albit), with a mean value of 56.62 mm. The best results were obtained when Albit fertiliser was used (mean 59.04 mm) and the worst in case of the control (mean 54.19 mm). No significant differences were recorded (p-0.1123).

Fruits of "Eureka" variety ranged in size from 55.42 mm (Cropmax) to 66.21 mm (Naturamin), with an average of 61.42 mm. The best results were obtained with Naturamin fertiliser (mean 63.07 mm) and the worst with Cropmax (mean 58.76 mm). No significant differences were recorded (p-0.2948).

Size of the fruits of "Elbertina" variety ranged from 46.50 mm (Cropmax) to 56.45 mm (Albit), with a mean of the experiment of 51.19 mm. The best results were obtained with Albit fertiliser (mean 53.40 mm) and the worst with Cropmax fertiliser (mean 48.34 mm). No significant differences were recorded (p-0.0856).

Fruits of "Desert Gold" variety ranged in size from 52.93 mm (Cropmax) to 61.55 mm (Albit), with a mean of 57.37 mm. The best results were obtained with Albit fertiliser (mean 58.32 mm) and the worst with Cropmax fertiliser (mean 54.92 mm). No significant differences were recorded (p-0.33).

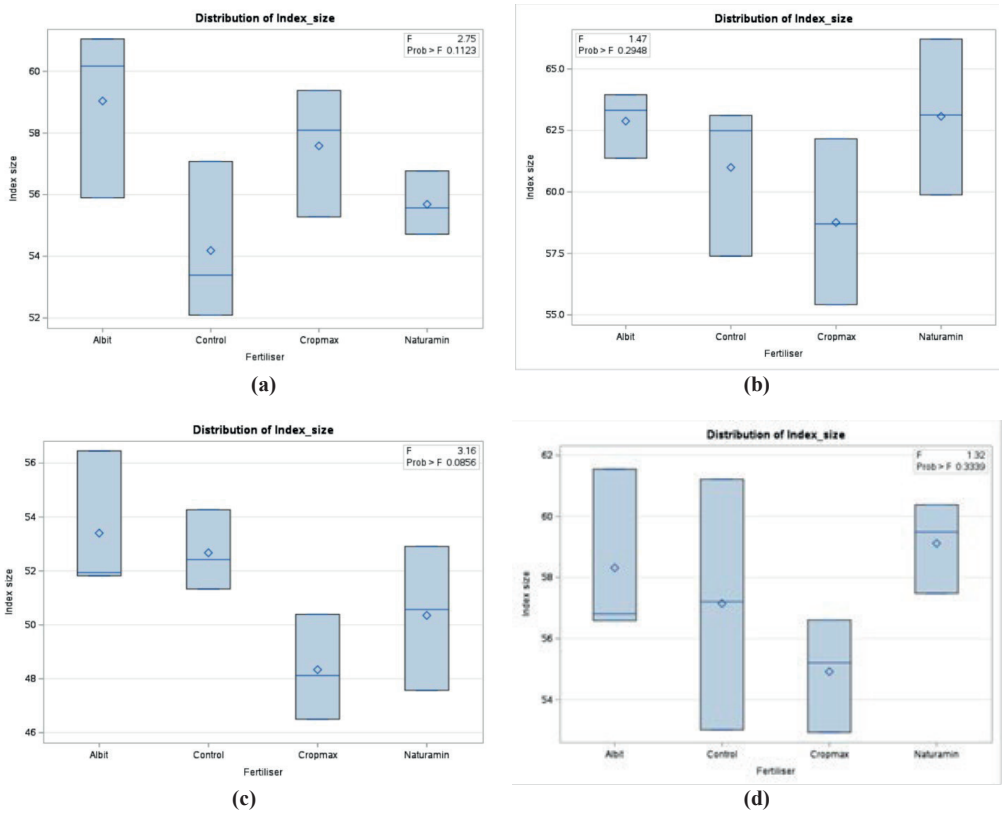


Figure 2. The influence of the fertilisers on the index size (mm)**:** (a) "Tokinostate"; (b) "Eureka"; (c) "Elbertina"; (d) "Desert Gold"

Fruit mass

The mass of the fruits of "Tokinostate" variety ranged from 109.40 g (Control) to 149.80 g (Cropmax), with a mean of 125.03 g.

The highest values were recorded when Cropmax fertiliser was applied (mean 133.30 g) and the lowest in case of the control (mean 116.60 g), with no significant differences recorded (p-0.3551).

Fruits from "Eureka" variety had a mass ranging from 100.50 g (Cropmax) to 185.50 g (Naturamin), with an experiment mean of 149.66 g. The highest values were obtained with Naturamin fertiliser (mean 157.77 g) and the lowest with Cropmax (mean 125.83 g). There were no significant differences recorded (p-0.1646).

The fruit mass of "Elbertina" variety ranged between 72.40 g (Cropmax) and 113.60 g (Albit), with a mean of 87.325 g. The highest masses were obtained in case of Albit fertiliser (mean 95.77 g) and the lowest with Cropmax (mean 76.53 g). There were no significant differences recorded (p-0.1104).

"Desert Gold" peaches' mass ranged from 98.40 g (Cropmax) to 152.80 g (Control), with a mean of the experiment of 122.03 g. The highest results were obtained with Albit fertiliser (mean 129.10 g) and the lowest with Cropmax (mean 110.07 g). Significant differences were not recorded (p-0.4978).

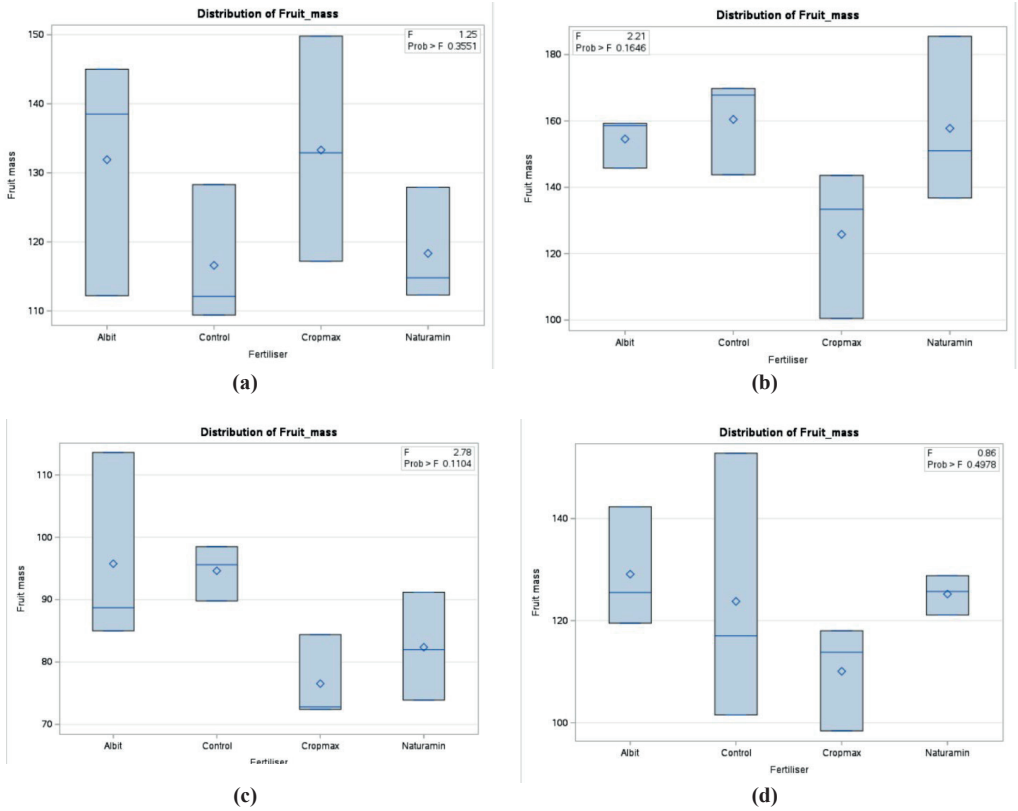


Figure 3. The influence of the fertilisers on the fruit mass (g): (a) "Tokinostate"; (b) "Eureka"; (c) "Elbertina"; (d) "Desert Gold"

Fruit hardness

"Tokinostate" fruits hardness ranged from 1255.00 kg/cm^2 (Naturamin) to 2501.00 kg/cm^2 (Control), with a mean of the experiment of 1977.25 kg/cm^2. The highest values were obtained in the control samples (mean 2233.67 kg/cm^2), and the lowest with Naturamin (mean 1500.67 kg/cm^2), but without significant differences (p- 0.0885).

The hardness of the fruits of "Eureka" variety ranged from 363.00 kg/cm^2 (Naturamin) to 851.00 kg/cm^2 (Albit), with a mean of the experiment of 554.66 kg/cm^2. The best results were obtained with Albit fertiliser (mean 693.33 kg/cm^2) and the worst with Naturamin (mean 374.33 kg/cm^2). Significantly positive differences were recorded for Albit and Cropmax samples compared to Naturamin and the control.

Fruits of "Elbertina" variety had hardness ranging from 340.00 kg/cm^2 (Naturamin) to 696.00 kg/cm^2 (Albit), with a mean of the experiment of 466.50 kg/cm^2. The highest values were recorded with Albit fertiliser (mean 574.33 kg/cm^2) and the lowest with Naturamin (mean 414.00 kg/cm^2). There were no significant differences recorded (p-0.1763).

Fruit hardness of "Desert Gold" variety ranged from 306.00 kg/cm^2 (Albit) to 1507.00 kg/cm^2 (Cropmax), with a mean of the experiment of 689.67 kg/cm^2.

The highest values were obtained with Cropmax fertiliser (mean 862.00 kg/cm^2) and the lowest with Albit (mean 418.00 kg/cm^2), with no significant differences (p-0.4272).

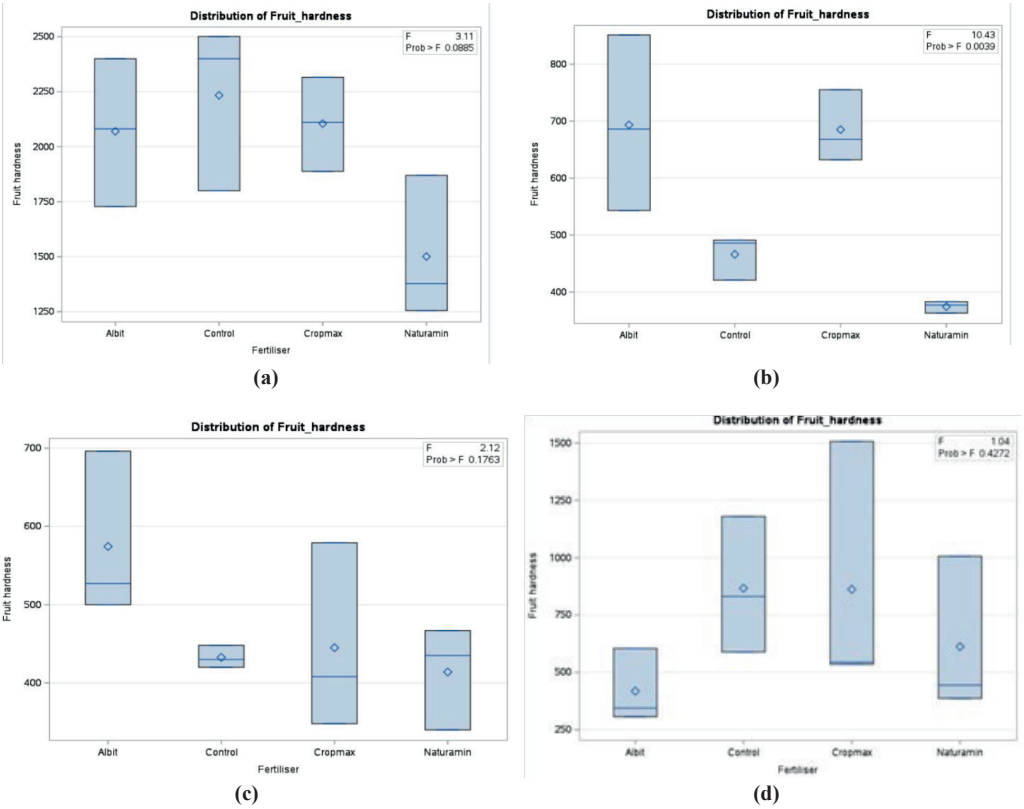


Figure 4. The influence of the fertilisers on the fruit hardness (kg/cm^2): (a) "Tokinostate"; (b) "Eureka"; (c) "Elbertina"; (d) "Desert Gold"

Soluble solid content

The soluble solid content of "Tokinostate" fruits ranged from 15.10 %Brix (Naturamin) to 22.70 %Brix (Cropmax), the average of the experiment being 20.83 %Brix. The highest values were recorded with Cropmax fertiliser (mean 21.93 %Brix) and the lowest with Naturamin fertiliser (19.53 %Brix), but without significant differences (p-0.4101).

The soluble solid content of the fruits of "Eureka" variety ranged from 10.30 %Brix (Control) to 12.90 %Brix (Albit), the mean of the experiment being 11.37 %Brix. The highest values were recorded with Albit fertiliser (mean 11.60 %Brix) and the lowest in case of the control (10.73 %Brix), but without significant differences (p-0.4898).

The soluble solid content of "Elbertina" fruits ranged from 17.90 %Brix (Control) to 22.70 %Brix (Albit), with the mean of the experiment of 20.02 %Brix. The highest values were recorded in the fruits treated with Albit fertiliser (mean 21.97 %Brix) and the lowest in the control samples (18.37 %Brix). Significantly positive differences were recorded for Albit and Cropmax variants compared to Naturamin treatments and to the control.

The soluble solid content of "Desert Gold" fruits ranged between 15.00 %Brix (Control) and 23.10 %Brix (Cropmax), the average value of the experiment being 18.99 %Brix. The highest values were recorded with the Cropmax fertiliser (mean 22.10 %Brix) and the lowest in the control (16.20 %Brix). Significantly positive differences were recorded for two variants (Cropmax and Albit), compared to the control and Naturamin variants.

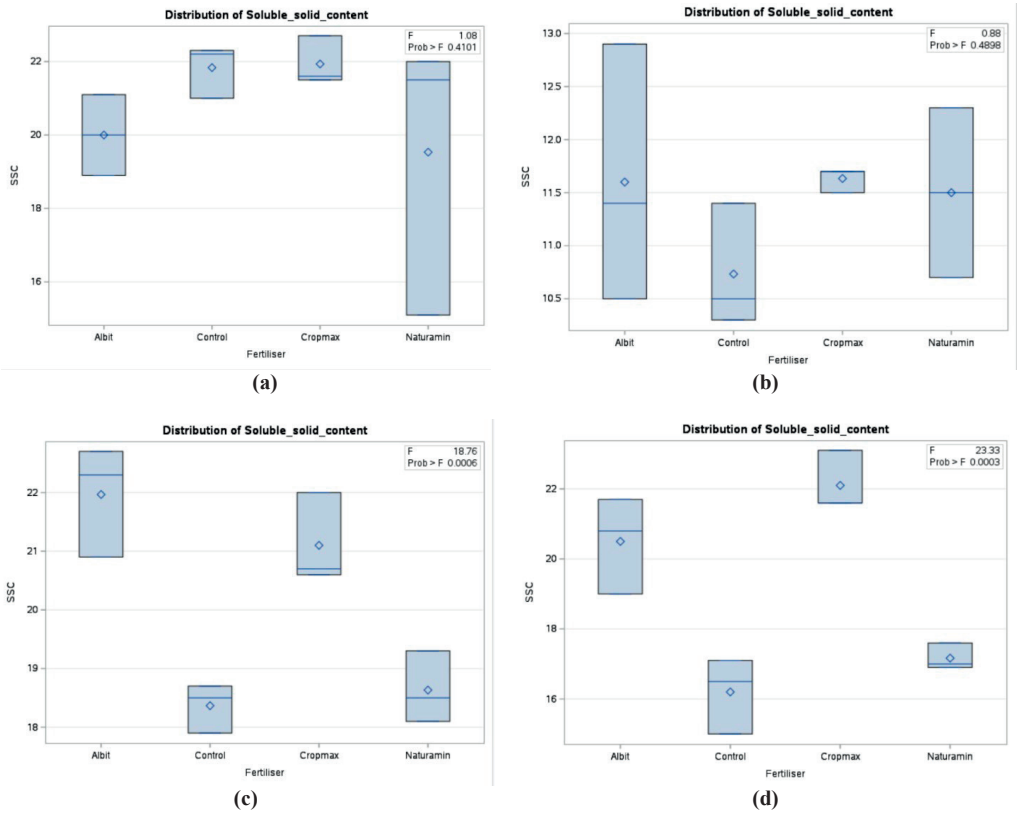


Figure 5. The influence of the fertilisers on the soluble solid content (%Brix): (a) "Tokinostate"; (b) "Eureka"; (c) "Elbertina"; (d) "Desert Gold"

Stone percentage

"Tokinostate" fruits had a stone percentage ranging from 5.21% (Cropmax) to 7.13% (Control), with an average of 6.1%. The lowest stone percentage was recorded when using Cropmax fertiliser (5.26%) and the highest in case of the control (6.85%). Significantly positive differences were recorded for Cropmax and Naturamin fertilisers compared to the control of the experiment.

Fruits of the "Eureka" variety had their stone percentage values ranging from 5.23% (Naturamin) to 8.76% (Cropmax), with an experiment mean of 6.72%. The lowest stone percentage was recorded for Naturamin fertiliser (5.80%) and the highest for Cropmax fertiliser (7.51%). No significant differences were recorded (p-0.0684).

The stone percentage of "Elbertina" fruits ranged from 3.14% (Control) to 5.36% (Cropmax), with an average of 4.32%. The smallest stone was recorded in the control (3.35%), and the highest when Cropmax fertiliser was used (5.09%). There were significantly positive differences for the control of the experiment in comparison with Cropmax fertiliser.

"Desert Gold" fruits had stone percentage values ranging from 4.74% (Naturamin) to 6.91% (Cropmax), with an experiment mean of 5.83%. The best results were recorded for Naturamin (5.12%) and the worst for Albit (6.38%). There were no significant differences.

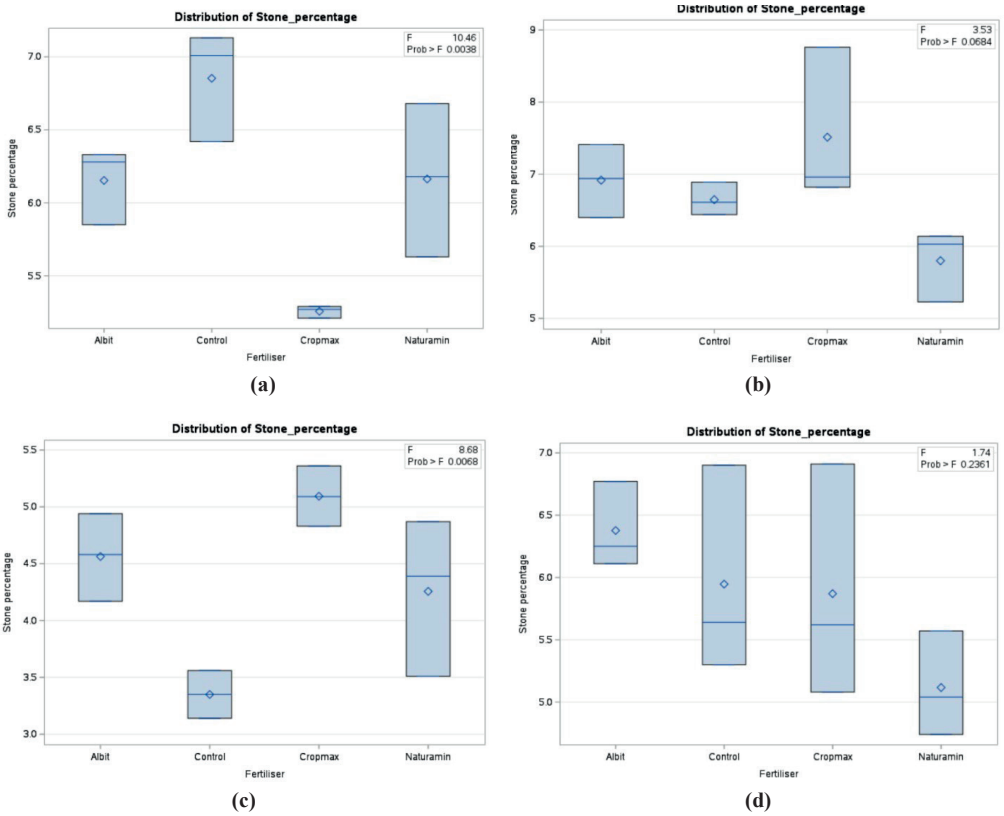


Figure 6. The influence of the fertilisers on the stone percentage (%): (a) "Tokinostate"; (b) "Eureka"; (c) "Elbertina"; (d) "Desert Gold"

Fruit yield per tree

The fruit yield per tree of "Tokinostate" variety ranged from 22.11 kg/tree (Control) and 59.52 kg/tree (Albit), with an average of the experiment of 35.07 kg/tree. The best values were recorded when using Albit fertiliser (46.28 kg/tree) and the worst results when Naturamin fertiliser was applied (25.98 kg/tree). Significantly positive differences were recorded for Albit fertiliser compared to Naturamin fertiliser.

The fruit yield per tree in case of "Eureka" ranged from 39.90 kg/tree (Control) to 45.22 kg/tree (Naturamin), with an average of 42.05 kg/tree. The best values were recorded with Naturamin fertiliser (42.84 kg/tree), and the worst results in the control (41.07 kg/tree). There were no significant differences recorded.

In case of "Elbertina" variety, the fruit yield per tree ranged from 32.24 kg/tree (Cropmax) and 49.20 kg/tree (Control), with a 41.48 kg/tree average. The best values were recorded in the control (48.00 kg/tree) and the worst for Cropmax fertiliser (35.73 kg/tree). Significantly positive differences were recorded for the control variant, and Albit fertiliser compared to Cropmax fertiliser, and for the control compared to Naturamin.

"Desert Gold" variety had a fruit yield per tree interval ranging from 21.90 kg/tree (Control) to 69.62 kg/tree (Cropmax), with an average of the experiment of 45.47 kg/tree. The highest values were recorded with the Cropmax fertiliser (60.25 kg/tree), and the lowest results with the control (23.23 kg/tree). There were significantly positive differences for Cropmax, Naturamin and Albit fertilisers compared to the control. Significantly positive differences were also recorded for Cropmax compared to Albit fertiliser.

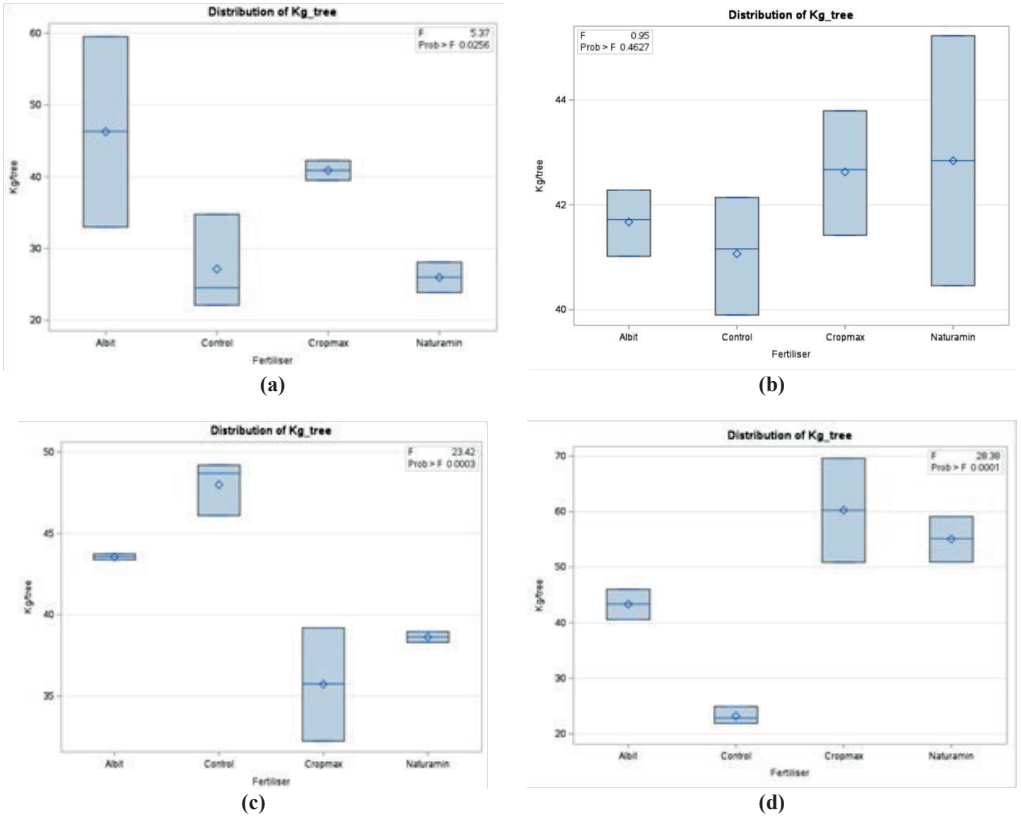


Figure 7. The influence of the fertilisers on fruit yield per tree (kg/tree): (a) "Tokinostate"; (b) "Eureka"; (c)"Elbertina"; (d) "Desert Gold"

CONCLUSIONS

Each fertiliser had different influences on the analyzed parameters of the four peach varieties. Statistically significant results were recorded for hardness, soluble solid content, stone percentage and yield per tree. As for the other two parameters, size index and fruit mass, even though statistically significant results were not recorded, the highest values, for all varieties, were obtained when fertilisers were used.

For hardness, significant differences were recorded only for "Eureka" variety, with positive influences of Albit and Cropmax fertilisers in comparison with the control, but also with Naturamin fertiliser.

Soluble solid content was improved in most of the varieties when using organic fertilisers. Statistically significant results were recorded in "Elbertina" and "Desert Gold" varieties, with positive influence of Albit and Cropmax fertilisers in comparison with the control and Naturamin fertiliser.

Regarding stone percentage, significant results were recorded for "Tokinostate" and "Elbertina" varieties. In case of "Tokinostate", Cropmax and Naturamin fertilisers had a significant influence compared to the control, the lowest stone percentage being obtained when using these fertilisers. In "Elbertina" variety, the lowest stone percentage was obtained in the control, with significant differences between it and Cropmax fertiliser.

In terms of yield per tree, statistically significant results were recorded for "Tokinostate", "Elbertina" and "Desert Gold" varieties, but the most representative results were obtained for "Desert Gold" variety, in which the use of Cropmax fertiliser resulted in almost three times the yield of the control.

Further studies are required, particularly concerning how fertilisers influence the internal properties of the fruits.

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THE INFLUENCE OF ORGANIC AND CONVENTIONAL CULTIVATION SYSTEMS ON SOME QUALITY AND PRODUCTIVITY INDICES OF THREE PEACH VARIETIES GROWING IN SOUTHEASTERN ROMANIA

Andreea Mihaela UDREA-BRASLĂ[1], Mihaela OLARU[1], Cătălin-Viorel OLTENACU[1], Nicoleta OLTENACU[1, 2], Ailin MOLOȘAG[1], Damian DRAGOMIR[1], Vlăduț HOLT[1]

[1]Research and Development Station for Fruit Tree Growing Băneasa,
8 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, 011464, Bucharest, Romania

Corresponding author: postolici.mihaela@scdpbaneasa.ro

Abstract

The peach (Prunus persica L.), native to China, is a fruit tree of global importance, appreciated for its nutritional and sensory qualities. This study analyzes the influence of organic and conventional cultivation technology on some fruit quality indices (firmness, sugar content, acidity, pH) and productivity of three peach varieties - 'Catherine Sel1', 'Springcrest' and 'Raluca' - grown in south-eastern Romania. The results showed that the conventional system significantly influenced the fruit weight, 61.64 g compared to 37.36 g in the organic system, and consequently the productivity was higher in the 'Catherine Sel1' variety. The fruit firmness was similar for both systems, while the sugar content was higher in fruit from conventional culture (17.3 °BRIX compared to 15 °BRIX). The pH values were comparable, with the 'Raluca' variety recording the highest pH (3.76) and the citric acid content showed minor differences between the systems (0.97% vs. 0.91%), with the 'Springcrest' variety reaching the highest value of 1.78%.

Key words*: organic system, conventional system, quality indices, peach, productivity.*

INTRODUCTION

The peach (*Prunus persica* L.) is one of the most important fruit species globally, valued for its nutritional and sensory qualities, such as its high vitamin, mineral and antioxidant content. Native to China, the fruit spread rapidly to temperate regions of the world and is now cultivated in many areas, including south-eastern Europe. In Romania, peach is an important crop with significant economic value in the agricultural sector. Peaches (*Prunus persica* L.) are the third most produced fruit in the world after pears and apples and have a huge domestic and export market. Peaches are rich in various nutritional compounds including vitamins, minerals, carbohydrates, organic acids and antioxidants (Naqash & Naik, 2022).

At the same time, the interest in sustainable agriculture has led to the development and application of organic farming practices, and the comparison of organic and conventional farming systems has become an important research topic in fruit growing. While organic farming is generally less productive than conventional farming, it can still produce higher quality fruit. This suggests that the focus should not only be on quantity, but also on quality (Munné-Bosch & Bermejo, 2024).

MATERIALS AND METHODS

The present study aims to evaluate the influence of organic and conventional cultivation systems on some relevant parameters of fruit quality and productivity, analyzing three peach varieties: 'Catherine Sel1', 'Raluca' and 'Springcrest' found in south-eastern Romania.

Figure 1 shows the 'Catherine Sel 1' peach variety, developed in 2001 at the Research and Development Station for Fruit Growing in Constanța (SCDP). It originated through the self-pollination of the American variety 'Catherine', followed by a careful selection of hybrid progenies. This variety is the first clingstone peach for the industry. The variety has been tested in comparative competition crops at Valu lui Traian and the University of Life Sciences in Bucharest, demonstrating outstanding performance in the pedoclimatic

conditions of Romania. The variety is characterized by medium-sized fruits, weighing between 80-110 grams, spherical in shape with a deep, pointed top and narrow and medium to deep peduncular cavity. The skin is medium-thick with dense, fine pubescence and a greenish-yellow ground color with reddish tints in the sunny areas of the fruit, giving it an attractive appearance. The pulp is yellow-orange, firm, rubbery, free of fibers and reddish infiltrations, with a pleasant taste and flavor that intensifies during processing, making it ideal for processing.



Figure 1. Catherine Sel. 1 variety
(Source: SCDP Constanta website)

The variety has a high sugar content (11-13% expressed refractometrically) and does not disintegrate on boiling. On the tree side, 'Catherine Sel1' is frost-hardy and tolerant to the main fruit diseases, such as leaf blight and leaf spot. Being a self-fertile variety, it does not require a pollinator and is characterized by a high vigour, which ensures a constant and very high yield of about 35-40 kg/tree, which can reach 22-25 t/ha for a density of 625 trees/ha, respectively 30-33 t/ha for 833 trees/ha. The tree goes into economic fruiting in the third year after planting, which makes it an attractive choice for growers who want a fast and steady production of high-quality fruit (SCDP Constanta).

Figure 2 shows 'Springcrest', an American apricot variety developed in 1969, known for its high vigor and self-fertility. The fruits of this variety have an average weight of 65-75 g and are spherical in shape. The skin is predominantly yellow-orange, with a deep crimson red over almost the entire surface and a freckled appearance on the shaded side.



Figure 2. Springcrest variety
(Source: SCDP Constanta website)

The flesh is yellow in color, without infiltrations, with a firm, juicy and adherent to the stone. It has a pleasant, slightly acidic taste and is considered excellent quality. The fruit ripens between June 20-30 and is characterized by high productivity. Thanks to its characteristics, this variety of apricot is used both for fresh consumption and for industrial processing, being used in compotes, jams, nectars and juices.



Figure 3. Raluca variety
(Source: SCDP Constanta website)

Figure 3 presents the peach variety 'Raluca', approved in 2001 and also known as V.T. HP-03. This variety was developed at the Research and Development Station for Fruit Growing in Constanța (SCDP) through the hybridization of the 'Liana' and 'Melania Dumitru' varieties. Tested at Valu lui Traian and USAMV Bucharest, it was characterized by high productivity and fruit quality.

The fruits are medium-sized (100-110 g), rounded, slightly asymmetrical, with greenish-yellow and reddish - grey skin on 70% of the surface. The pulp is yellow-orange, juicy, without fiber and with a pleasant taste. The stone

is medium and non-sticky to the flesh. Harvest maturity occurs in the first two decades of July. The tree is resistant to frost, requires phyto-sanitary protection, flowers abundantly and is self-fertile. Productivity is high (30-35 kg/tree). The variety is suitable for fresh consumption and processing and is economically valuable due to the quality fruit and early ripening period. Recommended in all areas favorable to peach.

This research is important in view of the diversity of peach varieties and the significant differences between cropping systems, which can influence both the physico-chemical characteristics of the fruit and the yields obtained. In this context, emphasis is placed on fruit quality parameters such as firmness, sugar content, acidity and pH, which are essential factors in determining market quality and consumer preferences. Peaches, nectarines and plums are high in fiber and among the 12 most nutritious fruits, they are in the middle percentile (Olaru Mihaela et al., 2024).

Preliminary results of the study indicate that the conventional farming system produced significantly heavier and more productive fruit compared to the organic system. Notable differences were also observed in sugar content and acidity of the fruit, with an advantage of conventional cultivation in sugar concentrations.

These findings are key to understanding the impact of different cultivation methods on the quality and quantity of apple products, providing valuable information for farmers who want to optimize peach production and meet market demands. Comparative nutritional studies on organic versus conventional fruit, emphasise that conventional cultivation often results in higher fruit weight, yield, sugar content and acidity, which are crucial for understanding the impact of cultivation methods on fruit quality (Maciej Gąstoł, 2020).

Fruit quality parameters were analyzed (weight, sugar content, acidity, firmness, pH).

The land on which the experiment is located belongs to the Băneasa Research-Development Station for Fruit Growing, the peach plantation was established in 2019. The experiment was conducted using a 4 x 2 m planting scheme, corresponding to a density of 1,250 trees/ha. It falls into the super-intensive category, ensuring efficient use of space, high production and modern plantation management, including the possibility of mechanizing some technological works. The experiment was bifactorial, taking into account the following experimental factors:

- **Factor A - Variety**
  - a1 - Catherine Sel1
  - a2 - Raluca
  - a3 - Springcrest
- **Factor B - farming system**
  - b1 - conventional system
  - b2 - organic system

Analyses were performed on a sample of 20 fruits per variety. The determinations concerned: fruit weight, soluble solid contents, measured with the Hanna Instruments HI 96800 digital refractometer (BRIX%), pH, determined with the Hanna HI 700630 pH meter, titratable acidity, determined with the Hanna Instruments model 230V Minitrometer (%) and fruit firmness, measured with the Effegi Penetrometer with 8 mm diameter piston (kg/cm^2).

The determinations were performed on fruit from the 2024 production, production influenced by 2024-specific climatic conditions. The predominant soil in the area is a reddish luvisol, and reddish luvisols and stagnosols are found in depressional areas and the cracks of the land (Dragomir et al., 2023). High clay content can lead to challenges such as compaction and poor aeration, which can hinder root development and water infiltration (Dimitrov et al., 2017).

Table 1. Chemical properties of Moara Domnească soil (Source: Nicoleta Oltenacu et al., 2024)

Oriz.	Depth (cm)	Soil pH ui	Total content			
			Humuss %	N-NO_3%	P_2O_5%	K_2O%
Ap	0-20	5.27	2.46	0.135	0.159	1.65
A/B	20-45	5.20	2.3	0.120	0.130	1.05
Bt	45-150	5.10	2.17	0.100	0.108	0.99
Cca	150-200	5.35	1.85	0.07	0.115	1.23

Understanding soil physical and chemical properties is crucial for developing effective land management strategies, particularly in areas prone to erosion and degradation due to climate change (Prachowski et al., 2024).

Figure 4 illustrates the monthly maximum and minimum temperatures, showing significant variations between seasons.

Maximum temperatures reach highs in the summer months, with a peak of 39.40°C in July, followed by 37.70°C in June and 36.90°C in August. Lows over the same period remain positive but more moderate (14.00°C in June and July, 14.80°C in August).

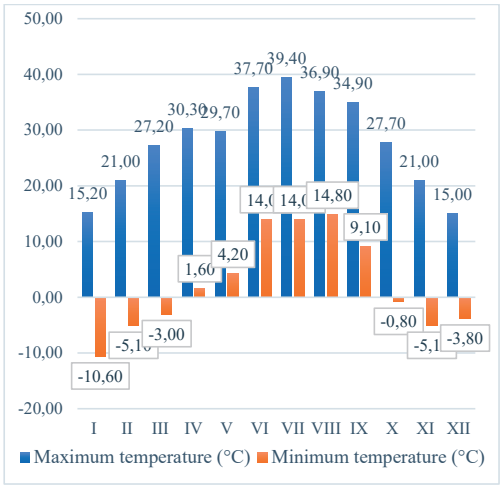


Figure 4. Distribution of maximum and minimum temperatures in 2024 (Source: Moara Domneasca Weather Station)

In winter, the contrast is strong, with highs ranging from 15.20°C in January to 21.00°C in February and November, but with extreme negative lows, reaching -10.60°C in January and -5.10°C in February and November.

These variations reflect a temperate-continental climate characterized by cold winters and very hot summers. In the context of climate change, there is a trend of increasing annual temperatures, with warmer summers and milder winters, which may affect agriculture and local ecosystems. Adapting to these changes becomes essential for managing the impact on the environment and the economy.

Figure 5 shows the monthly rainfall distribution, showing significant variations throughout the year. The total amount of precipitation recorded is 557.2 mm, but it is unevenly distributed, with peaks in March, November and December (above 80-120 mm) and lower values in summer and early autumn.

This uneven distribution can create major problems, such as periods of drought in the warm months, affecting agriculture and water supplies, followed by episodes of heavy rainfall in the cold months, which can lead to flooding and soil erosion.

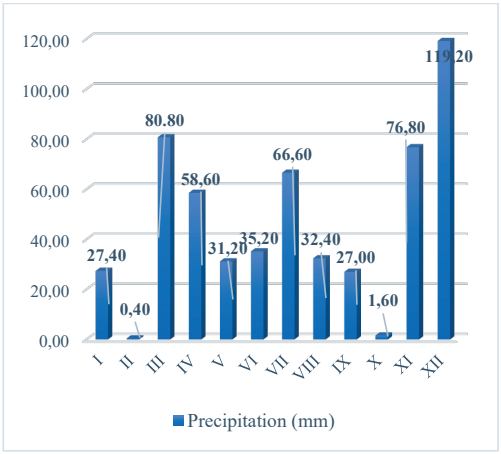


Figure 5. Distribution of precipitation in 2024 (Source: Moara Domnească Weather Station)

In the context of climate change, such variations are becoming more pronounced, requiring water management measures and adaptation of agricultural practices. Higher temperatures and extreme weather events such as droughts and floods reduce agricultural productivity, threatening food security (Eswaran et al., 2024). In hot summers, even if, as shown in the graph, there is precipitation, it is not properly distributed. Higher temperatures caused by climate change cause species to migrate, leading to biodiversity loss, while extreme weather events reduce crop productivity, threatening food security. Adaptation strategies, such as the development of climate-resilient crops, are key to managing these economic and environmental impacts (Mostafa et al., 2025). The amount of rainfall is insufficient during critical periods for crops, and due to high temperatures and soil type, evaporation is very intense. This prevents water from infiltrating and being used efficiently by the plants. To compensate for this problem, we have used irrigation, thus providing the necessary moisture for the plants and preventing production losses due to drought. Modern fruit growing cannot succeed without an adequate water regime, which is essential for the health and productivity of the fruit species. Irrigation is emphasized as a crucial tool for effective growth management of fruit trees (Oltenacu & Oltenacu, 2014).

RESULTS AND DISCUSSIONS

Table 2 presents the comparative analysis of the technological operations performed in the experimental peach batch studied, highlighting the differences between two production systems: the conventional system and the organic system.

Table 2. Technological works realized in this experimental lot

Active substance of PPP	Recommended dose	Required dose	Value lei
Copper sulfate 20%	5	2.50	197.37
Paraffin oil 60% Neem oil 10% Orange oil 2.5% Rapeseed oil 5% Vegetable oil (glycerol - inert material) 20%	100%	0.50	40.16
Fungicide and insect - acaricide Limocide	6	3	1151.32
Trichoderma atroviride (harzianum spp.); Trichoderma hamatum; Trichoderma koningii;	0.5	0.25	276.32
Bacillus thuringiensis subsp. Kurstaki - 2x109 cfu/ml Glycerol - 20% (inert material)	1	0.5	294.74
Copper hydroxide, Copper oxychloride	4	2	136.84
Paraffin oil 96.5 g/kg, formulation ingredients	1.5	0.75	131.58
copper 272 g/l (copper hydroxide 136 g/l	4	2	157.89
copper oxychloride 136 g/l	1.5	0.75	921.05
60% mimosa tenuiflora extract; - 20% citrus fruit extract.	3	1.5	807.89
Neem extract	3	1.5	455.26
Vegetable oils	3	1.5	368.42
Azadirachtin 10 g/l	3	1.5	1710.53
Paraffin oil 800 g/l	1.5	0.75	131.58
			Total lei: 6780.95

In the conventional system, the main interventions include mechanical or manual pruning, chemical fertilizer application, spraying with plant protection products, green pruning, tillage with a palpatory tiller and mechanical mowing**.** The system also involves mechanized operations such as servicing agricultural machinery and transporting materials. Instead, in the organic system, the technological work is adapted to agro-ecological principles, including hand weeding, crown-forming pruning, branch pruning, soil loosening, hand mowing and the use of alternative plant protection methods such as spraying with vermorel.

Table 3 shows the plant protection products used in treatments applied within the conventional cropping system.

Table 3. Chemical plant protection products applied to Peach

Active substance of PPP	Recommended dose	Dosage required	Amount lei
220–25% metallic copper, 10–12% soluble or organic sulfur	5	2.50	72.37
790 g/L paraffin oil.	1	0.50	52.63
Dodine	2	1.00	136.84
clorantraniliprol 200 g/l	0.18	0.09	10.53
difenoconazol 250 g/l	0.2	0.10	73.68
50 g/l lambda – cihalotrin	0.2	0.10	69.74
dodine 400 g/l	2.0	1.00	151.32
500 g/kg fenpira-zamine	1	0.50	657.89
Difenoconazol 250 g/L	0.2	0.10	55.26
250 g/l cipermetrin	0.2	0.10	39.47
difenoconazol 250 g/l	0.2	0.10	73.68
deltametrin (piretroid)	0.3	0.11	27.63
6,7 % Piraclostrobin, 26,7 % Boscalid	0.5	0.25	46.05
Lambda - cihalotrin 50 g/l	0.15	0.08	69.74
Luna fluopiram 200 g/L+tebuconazol 200 g/L400SC	0.5	0.25	85.53
spirotetramat 100 g/l	1.875	0.94	888.16
20% metallic copper and 80% neutralized copper sulfate	2.6	1.30	163.16
			Total lei: 2673.68

It includes active substances used to protect plants against diseases and pests, including compounds such as metallic copper, dodine, chlorantraniliprole, as well as various fungicides and insecticides. The total cost of applying phytosanitary treatments is 2673.68 lei.

Table 4 shows the organic plant protection products applied for the same species. These include alternative products based on natural ingredients such as copper sulphate, vegetable oils (paraffin, neem, orange, rapeseed), natural extracts and beneficial micro-organisms such as Trichoderma spp., Bacillus subtilis and Bacillus thuringiensis. This type of treatment aims at an integrated and sustainable protection of the crop, reducing the environmental impact. However, it is observed that the ecological treatment scheme involves a higher cost, with a total of 6780,95 lei, compared to the costs of phytosanitary treatments for the conventional system.

The analyzed data highlight the influence of the cultivation method (conventional or organic) on some physical and environmental parameters and fruit biochemicals for thee varieties: 'Catherine Sel 1', 'Raluca' and 'Springcrest'.

Table 4. ECO plant protection products applied to Peach species

Conventional system	**Organic system**
Disking	Crown-forming cuts
Removing dead trees	Thinning ground
Apply chemical fertilizers	Spraying plantations
Irrigated plantations	Servicing irrigation system
Green pruning	Deservit sistem irigat
Harvested apricots for internal consumption	Servicing agricultural machinery
Worked the soil with a rotary cultivator on the rows of trees	Worked the soil with a rotary tiller on rows of trees
Prepare solution for spraying	Various transportation
Serviced the spraying machine	Mechanical mowing
Mowed the trimmings (manually)	Loosened the soil between rows of trees
Serviced irrigation system	Plantation watering
Serviced agricultural machinery	Tilled the soil with a rotary cutter with feeler between rows of trees
Various transportation	Tractor + machinery maintenance
Administered chemical fertilizers	
Mechanical mowing	
Tractor + machinery maintenance	
Worked the soil with a rotary tiller on rows of trees	
Spraying plantations	

Table 5. Physical determinations of fruit by cropping system

Conventional system	**Organic system**
Row-harrowed with hoe	One by one digging
Administered chemical fertilizers	Weeding or suppressing shoots of young plantations
Pruned or suppressed shoots of young plantations	Weeding dead trees
Pinching shoots	Tightening branches in piles

Dependent Variable	**Factor**	**Mean Difference**	**Significance**	**Significance meaning**
Weight	a1b1-a1b2	23.4375	0	***
	a2b1-a2b2	7.5175	0.039	*
	a3b1-a3b2	8.6525	0.086	n.s
Firmness	a1b1-a1b2	1.1175	0.126	n.s
	a2b1-a2b2	0.33	0.49	n.s
	a3b1-a3b2	0.385	0.84	n.s.
Production	a1b1-a1b2	2.9325	0	***
	a2b1-a2b2	0.8925	0.378	n.s.
	a3b1-a3b2	0.9975	0.456	n.s.
Factor A- Varietal a1 -Catherine sel 1 a2 - Raluca a3 - Springcrest	Factorul B – culture system b1 - conventional b2 - ecological		n.s. - not significant * - significant ** - distinctly significant *** - highly significant	

Fruit weight is significantly influenced by the cropping system, being higher in the conventional system. Conventional systems often produce larger fruit, but organic methods can

increase firmness due to better soil management practices (Iancu & Gavat, 2009). For the variety 'Catherine Sel 1', the difference is very significant (+23.4375 g, p<0.001), which indicates a better development of this variety under the conditions offered by fertilization and treatments specific to conventional agriculture. Also for 'Raluca', fruit weight is significantly higher in the conventional system (+7.5175 g, p<0.05), although the difference is not as pronounced. However, 'Springcrest' shows no significant differences between the two systems (+8.6525 g, p=0.086), suggesting a stability of fruit mass whether grown under organic or conventional systems. On the other hand, fruit firmness is not influenced by the cropping system for any of the varieties. The values obtained for 'Catherine Sel 1' (+1.1175, p=0.126), 'Raluca' (+0.33, p=0.490) and 'Springcrest' (+0.385, p=0.840) show that this characteristic remains stable, regardless of whether the fruit is grown under conventional or organic farming.

In terms of fruit yield, 'Catherine Sel 1' stands out with a highly significant difference in favour of the conventional system (+2.9325 kg, p<0.001), indicating higher productivity when using conventional fertilization and crop protection methods. However, for 'Raluca' (+0.8925 kg, p=0.378) and 'Springcrest' (+0.9975 kg, p=0.456), the differences are not significant, suggesting that these varieties maintain their productivity whether they are grown under organic or conventional systems.

Table 6. Biochemical determinations of fruit by culture system

Dependent Variable	Factor	Mean Difference	Significance	Significance meaning
BRIX	a1b1-a1b2	0.82	0.046	*
	a2b1-a2b2	1.46	0.001	**
	a3b1-a3b2	0.29	0.196	n.s.
pH	a1b1-a1b2	0.0175	0,68	n.s.
	a2b1-a2b2	0.34	0	***
	a3b1-a3b2	0.1175	0.114	n.s.
Citric acid	a1b1-a1b2	-0.0075	0.717	n.s.
	a2b1-a2b2	-0.0875	0.048	*
	a3b1-a3b2	-0.135	0.031	*
Factor A- Varietal a1 -Catherine sel 1 a2 - Raluca a3 - Springcrest	Factor B - cropping system b1 - conventional b2 - organic		n.s. - not significant * - significant ** - distinctly significant *** - highly significant	

As for the sugar content, it is significantly higher in conventional cultivation for the variety 'Catherine Sel 1' (0.82, p<0.05), and for 'Raluca', the difference is even more pronounced, being distinctly significant (1.46, p<0.01). These results suggest that the conventional system favors carbohydrate accumulation in these two varieties. On the other hand, in 'Springcrest', the difference between the two cropping systems is not significant (0.29, p=0.196), indicating that this variety maintains its sugar levels regardless of the farming technology used.

The analysis of fruit pH shows that only 'Raluca' presents a very significant difference between the two cultivation systems, having a higher pH in conventional (0.34, p<0.001). In the case of the other varieties, 'Catherine Sel 1' (0.0175, p=0.680) and ‘Springcrest‘ (0.1175, p=0.114), the differences are not significant, which indicates that this characteristic remains constant regardless of the cultivation method.

At the citric acid level, the differences are significant only for the varieties ‘ Raluca‘ and ‘Springcrest‘ , where a decrease is observed in the conventional system. In ‘Raluca‘, the difference is significant (-0.0875, p<0.05), and in ‘Springcrest‘ , it is also significant (-0.135, p<0.05). In contrast, for ‘ Catherine Sel 1‘, no significant changes were observed between the two cultivation systems (-0.0075, p=0.717), suggesting that this variety has greater acidity stability. Organic peaches show higher titratable acidity and sugar levels compared to conventional ones, improving flavor profiles (Castro et al., 2014). Comparative interpretation of varieties according to the cropping system.

The comparative analysis of the three varieties, ‘Catherine Sel 1‘, ‘Raluca‘ and ‘Springcrest‘, within the two cultivation systems, conventional (b1) and organic (b2), highlights significant differences in terms of the physical and biochemical parameters of the fruits.

Table 7. Comparison of physical and biochemical parameters between varieties in the conventional system

Parameters	Variety	Mean Difference	Std. Error	Significance	Significance meaning
Weight	b1a1-b1a2	7.3575	4.38943	0.266	n.s.
	b1a1-b1a3	4.6675	4.38943	0.559	n.s.
	b1a2-b1a1	-7.3575	4.38943	0.266	n.s.
	b1a2-b1a3	-2.69	4.38943	0.817	n.s.
	b1a3-b1a1	-4.6675	4.38943	0.559	n.s.
	b1a3-b1a2	2.69	4.38943	0.817	n.s.
Firmness	b1a1-b1a2	5.62500(*)	1.5605	0.014	*
	b1a1-b1a3	-2.955	1.5605	0.196	n.s.
	b1a2-b1a1	-5.62500(*)	1.5605	0.014	*
	b1a2-b1a3	-8.58000(*)	1.5605	0.001	**
	b1a3-b1a1	2.955	1.5605	0.196	n.s.
	b1a3-b1a2	8.58000(*)	1.5605	0.001	**
BRIX	b1a1-b1a2	0.19	0.28207	0.784	n.s.
	b1a1-b1a3	6.28000(*)	0.28207	0	***
	b1a2-b1a1	-0.19	0.28207	0.784	n.s.
	b1a2-b1a3	6.09000(*)	0.28207	0	***
	b1a3-b1a1	-6.28000(*)	0.28207	0	***
	b1a3-b1a2	-6,09000(*)	0.28207	0	***
pH	b1a1-b1a2	-0.17750(*)	0.04233	0.006	**
	b1a1-b1a3	-0.0875	0.04233	0.152	n.s.
	b1a2-b1a1	0.17750(*)	0.04233	0.006	**
	b1a2-b1a3	0.09	0.04233	0.139	n.s.
	b1a3-b1a1	0.0875	0.04233	0.152	n.s.
	b1a3-b1a2	-0.09	0.04233	0.139	n.s.
Citric acid	b1a1-b1a2	0.16250(*)	0.04683	0.017	*
	b1a1-b1a3	-0.93000(*)	0.04683	0	***
	b1a2-b1a1	-0.16250(*)	0.04683	0.017	*
	b1a2-b1a3	-1.09250(*)	0.04683	0	***
	b1a3-b1a1	0.93000(*)	0.04683	0	***
	b1a3-b1a2	1.09250(*)	0.04683	0	***
Production	b1a1-b1a2	1.3525	1.25267	0.549	n.s.
	b1a1-b1a3	1.66	1.25267	0.417	n.s.
	b1a2-b1a1	-1.3525	1.25267	0.549	n.s.
	b1a2-b1a3	0.3075	1.25267	0.967	n.s.
	b1a3-b1a1	-1.66	1.25267	0.417	n.s.
	b1a3-b1a2	-0.3075	1.25267	0.967	n.s.
Factor A- Variety a1 -Catherine sel 1 a2 - Raluca a3 - Springcrest	Factor B - cropping system b1 - conventional b2 - organic		n.s. - not significant * - significant ** - distinctly significant *** - highly significant		

Fruit weight analysis does not reveal significant differences between the studied varieties, indicating a comparable fruit mass, regardless of the variety.

Regarding fruit firmness, the ‘Springcrest‘ variety presents a significantly higher consistency than 'Raluca' (8.5800, $p<0.01$), suggesting a firmer tissue structure under conventional cultivation conditions.

The evaluation of the biochemical composition highlights notable variations between genotypes. ‘Catherine Sel 1‘ and 'Raluca' register a significantly higher sugar content compared to ‘Springcrest‘ (6.2800 and 6.0900, $p<0.001$). Regarding acidity, ‘Springcrest‘ presents the highest concentration of citric acid, with significant differences compared to the other varieties analyzed (1.0925, $p<0.001$). At the same time, the pH values are slightly higher in 'Catherine Sel 1' compared to 'Raluca' (-0.1775, $p<0.01$), which suggests a slightly lower acidity for this genotype. In terms of productivity, no significant differences are evident between the varieties, which indicates a comparable productive potential of these varieties in the conventional cropping system.

Table 8. Comparison of physical and biochemical parameters between varieties in the ecological system

Parameters	Variety	Mean Difference	Std. Error	Significance	Significance meaning
Weight	b2a1-b2a2	-8.56250(*)	1.09172	0	***
	b2a1-b2a3	-10.11750(*)	1.09172	0	***
	b2a2-b2a1	8.56250(*)	1.09172	0	***
	b2a2-b2a3	-1.555	1.09172	0.37	n.s.
	b2a3-b2a1	10.11750(*)	1.09172	0	***
	b2a3-b2a2	1.555	1.09172	0.37	n.s.
Firmness	b2a1-b2a2	4.83750(*)	0.4307	0	***
	b2a1-b2a3	-3.68750(*)	0.4307	0	***
	b2a2-b2a1	-4.83750(*)	0.4307	0	***
	b2a2-b2a3	-8.52500(*)	0.4307	0	***
	b2a3-b2a1	3.68750(*)	0.4307	0	***
	b2a3-b2a2	8.52500(*)	0.4307	0	***
BRIX	b2a1-b2a2	0.83000(*)	0.23458	0.016	**
	b2a1-b2a3	5.75000(*)	0.23458	0	***
	b2a2-b2a1	-0.83000(*)	0.23458	0.016	**
	b2a2-b2a3	4.92000(*)	0.23458	0	***
	b2a3-b2a1	-5.75000(*)	0.23458	0	***
	b2a3-b2a2	-4.92000(*)	0.23458	0	***
pH	b2a1-b2a2	.14500(*)	0.04856	0.037	*
	b2a1-b2a3	0.0125	0.04856	0.964	n.s.
	b2a2-b2a1	0.,14500(*)	0.04856	0.037	*
	b2a2-b2a3	-0.1325	0.04856	0.055	n.s.
	b2a3-b2a1	-0.0125	0.04856	0.964	*
	b2a3-b2a2	0.1325	0.04856	0.055	n.s.
Citric acid	b2a1-b2a2	0.08250(*)	0.02118	0.009	**
	b2a1-b2a3	-1.05750(*)	0.02118	0	***
	b2a2-b2a1	-0.08250(*)	0.02118	0.009	**
	b2a2-b2a3	-1.14000(*)	0.02118	0	***
	b2a3-b2a1	1.05750(*)	0.02118	0	***
	b2a3-b2a2	1.14000(*)	0.02118	0	***
Production	b2a1-b2a2	-0.6875	0.27502	0.079	n.s.
	b2a1-b2a3	-0.275	0.27502	0.595	n.s.
	b2a2-b2a1	0.6875	0.27502	0.079	n.s.
	b2a2-b2a3	0.4125	0.27502	0.336	n.s.
	b2a3-b2a1	0.275	0.27502	0.595	n.s.
	b2a3-b2a2	-0.4125	0.27502	0.336	n.s.
Factor A- Variety a1 -Catherine sel 1 a2 - Raluca a3 - Springcrest	Factor B - cropping system b1 - conventional b2 - organic		n.s. - not significant * - significant ** - distinctly significant *** - very significant		

In the ecological culture, the 'Springcrest' variety is noted for the firmness of the fruits. Also, the firmness of the fruits is the highest at 'Springcrest' (8.5250, $p < 0.001$), which confirms the tendency observed in the conventional system.

The weight of the fruits remains similar between varieties, without significant differences. At the biochemical level, ‘Springcrest‘ has a significantly higher sugars compared to ‘Catherine Sel 1‘ and ‘Raluca‘ (5.7500, $p < 0.001$), which suggests that this variety accumulates carbohydrates in the ecological system. Also, the acidity is the highest at ‘Springcrest‘, having the highest citric acid content (1.1400, $p < 0.001$), which can influence the taste of fruits.

As for the pH of the fruits, the differences are less pronounced, but ‘Catherine Sel 1‘ has a slightly higher pH compared to ‘Springcrest‘ (-0.1450, $p < 0.05$), which indicates a lower acidity to this variety. In terms of production, there are no significant differences between varieties, which suggests that, similar to conventional culture, all three varieties have a comparable productive potential.

Table 9 presents a comparison of the costs associated with peach cultivation in the two cultivation systems: conventional and organic.

Tabel 9. Peach cost analysis: conventional vs. ecological

Conventional system	Price (lei)	Ecological system	Price (lei)
Mechanical works	2885.75	**Mechanical works**	6378.33
Manual works	19254.8	**Manual works**	22578
Fertilizers NPK-16.16.16	1280	**Fertilizers NPK-16.16.16**	0
Phytosanitary products	2673.68	**Phytosanitary products**	6780.95
Diesel fuel	269.08	**Diesel fuel**	195.12
Diesel cost	2098.82	**Diesel cost**	1521.94
Total/lei	25788.4	**Total/lei**	30673.3

According to Table 9, the total costs related to peach cultivation in the conventional system are 25,788.4 lei, while for the organic system they reach 30,673.3 lei, which indicates an increase of approximately 22% in expenses in the case of the organic method.

The main cost differences are noted in the following categories:

Mechanical work is significantly more expensive in the organic system (6,378.33 lei) compared to the conventional one (2,885.75 lei). This difference is generated by the need for more frequent and more intensive interventions to maintain the health of the plantation in the absence of chemical pesticides.

Manual work involves a higher cost in the organic system (22,578 lei) compared to the conventional one (19,254.8 lei), which reflects the additional effort required to maintain the trees and apply biological treatments.

Phytosanitary products represent a category of costs that varies considerably: in the conventional system they are 2,673.68 lei, and in the organic system they reach 6,780.95 lei. The difference is due to the use of organic products, which are more expensive, but also to the need for their more frequent application.

Fertilizers represent an exclusive expense for the conventional system. Complex fertilizers of the NPK-16.16.16 type were applied, worth 1280 lei, while in the organic system no synthetic chemical fertilizers were used.

The data in Figure 6 indicate that the conventional system provides higher production, while the organic system compensates with a higher selling price. Conventional systems are usually more productive due to the use of chemical inputs, but organic systems offer economic benefits through higher prices for organic fruits. Conventional systems usually achieve higher yields, but at the cost of environmental degradation (Devilliers et al., 2023). In the conventional system, production varies between 3,881.25 kg/0.5 ha 'Springcrest' and 4,962.5 kg/0.5 ha 'Raluca'. At a price of 5 lei/kg, the maximum economic value achieved is 24,500 lei for 'Raluca' and 19,406.25 lei for 'Springcrest'. In the organic system, production is lower, ranging between 3,256.25 kg/0.5 ha 'Springcrest' and 3,512.5 kg/0.5 ha 'Raluca'. However, due to a higher price of 10 lei/kg, the maximum economic value achieved is 35,125 lei for 'Raluca' and 32,562.5 lei for 'Springcrest'. Organic systems may have lower immediate yields, but can provide long-term economic benefits through sustainable practices and reduced input costs (Nakhaii et al., 2024).

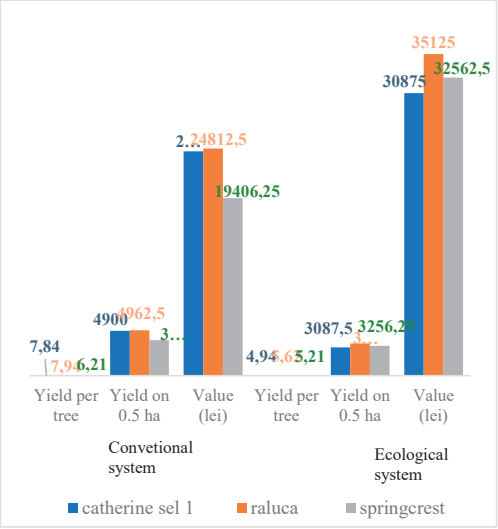


Figure 6. Production recorded in conventional and organic systems

Thus, the conventional system is more productive, but the organic system offers an economic advantage through higher fruit prices. The choice of cultivation method depends on the farmer's economic objectives and access to markets for organic fruit.

CONCLUSIONS

The comparative analysis of the two cultivation systems, conventional and organic, highlights significant differences in terms of both production and economic value of the fruits. The conventional system ensures a higher yield, due to the use of chemical fertilizers and synthetic

phytosanitary products, with a maximum production of 4,962.5 kg/0.5 ha 'Raluca'. In contrast, the organic system generates a lower production, but the higher selling price of organic fruits compensates for this disadvantage, leading to a higher economic value, reaching 35,125 lei for 'Raluca' at a price of 10 lei/kg.

From a qualitative point of view, fruits from the organic system have a higher sugar content and a more pronounced acidity in certain varieties, which can positively influence the organoleptic profile. Production costs are higher in the organic system, with an increase of approximately 22% compared to the conventional system, due to intensive manual work and the use of alternative plant protection products.

Thus, the choice of crop system depends on the farmer's economic objectives and market requirements. The conventional system maximizes productivity and economic efficiency in the short term, while the organic system offers superior financial benefits through premium fruit prices, along with a reduced ecological impact and sustainable agricultural practices.

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CURRENT STATUS OF GRAPEVINE FLAVESCENCE DORÉE AND BOIS NOIR IN BULGARIA (2022-2024)

Zhelyu AVRAMOV, Iliyana ATANASOVA

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: iatanasova@ltu.bg

Abstract

The present article examines the monitoring of phytoplasma diseases in grapevines during the period 2022-2024, with a special focus on the distribution, symptoms, diagnosis, and control methods of these diseases. Phytoplasma diseases, such as Flavescence dorée and Bois noir, pose a serious risk to viticulture due to the significant economic losses they cause. Molecular methods were used in the study to detect phytoplasmas. A total number of samples was more than 660 were analyzed, of which around 6 indicated a positive result for the phytoplasma disease Bois noir. And for the time being, the phytoplasma disease Flavescence dorée is not present in Bulgaria. Regardless of the status of the two phytoplasmas, from quarantine to non-quarantine controlled, according to phytosanitary legislation, the risk of their spread poses a significant threat to viticulture in Bulgaria. The obtained results highlight the need for integrated control strategies, including early diagnosis, vector control, and the use of resistant grapevine varieties, to prevent the introduction of this phytoplasma disease.

Key words*: Phytoplasma diseases, Flavescence dorée, Bois noir, Bulgaria.*

INTRODUCTION

Viticulture is a key subsector in Bulgarian agriculture, ranking second in monetary terms after tobacco production (Avramov & Mihaylov, 2019). Following this period, it is positioned directly after fruit growing and vegetable production (Agrostat, 2021). Despite its economic importance, viticulture faces numerous challenges, including grapevine yellows - a widespread global issue representing serious diseases transmitted through infected planting material. The diversity of symptom manifestations leads to the naming of various phytoplasma diseases in grapevines (Avramov, 2019). Phytoplasmas are submicroscopic bacteria lacking a cell wall, characterized by high pleomorphism and the absence of flagella or spores. They infect the plant's vascular system, primarily affecting the phloem. This results in severe disruptions to cellular structures, including the destruction of chloroplasts and reduced photosynthetic activity. In natural conditions, the transmission of phytoplasmas occurs primarily through cicadas and leafhoppers. These pathogens multiply in the body cavity and hemolymph of their vectors. The vectors can transmit the pathogen after feeding or within a few hours to days. In Bulgaria, data on phytoplasma diseases in grapevine plantations are scarce and do not meet modern scientific research standards (Dobrev, 1909; Trifonova et al., 1952; Abrascheva, 1977; Kovachevski et al., 1999; Avramov et al., 2007; Avramov et al., 2018). Grapevine yellows (GY), caused by various phytoplasmas worldwide, cannot be diagnosed based solely on symptoms. The most severe form of this disease, Flavescence dorée (FD), causes significant damage in major viticultural regions in France, Italy, Spain, Slovenia, Croatia, and Serbia (Belli, 2010). In Bulgaria, the FD phytoplasma is classified as a quarantine pest. Another important type of GY in Europe is Bois noir (BN), associated with the stolbur phytoplasma (STOL) or 16SrXII, which is primarily transmitted by the cicada *Hyalestes obsoletus* (Avramov et al., 2007; Avramov et al., 2011). The hosts of the Grapevine flavescence dorée phytoplasma include *Vitis vinifera* and other species and hybrids of the genus *Vitis* (Eveillard et al., 2016). Its spread has been reported in eight major EU member states involved in viticulture, such as Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia, and Spain, as well as in Switzerland and Serbia

(Jeger et al., 2016). The most effective vector for transmitting this phytoplasma is *Scaphoideus titanus* (Mori et al., 2002; Schvester et al., 1963). The vector of Grapevine flavescence dorée, *Scaphoideus titanus*, originating from North America (Papura et al., 2012), is distributed in 18 European countries (Jeger et al., 2016). The phytoplasma is transmitted to a new host plant through the insect's saliva (Wang et al., 2024). Symptoms typically become more pronounced during the summer months. In some cases, individual shoots of the vine may be affected, while in others, the entire vine shows signs of infection (Belli et al., 1973; 2010; Caudwell, 1964). In cases of late infection, the process of wood formation in the vine stops. The leaves undergo changes in color, and their edges curl downward. If the infection occurs early in the season, reduced fruit formation is observed, with inflorescences drying up and falling off. In later infections, the grape clusters turn brown and shrivel, and their stems dry out. The spread of Flavescence dorée in grapevines occurs primarily through two means: the transfer of infected plant material and the dispersal of infected vectors that migrate from neighboring areas, carried by planting material or through transportation. In 1961, a disease of the "yellows" type was first recorded in the viticultural regions of northeastern France, known as "Bois noir" (BN). A few years later, similar symptoms were observed in Germany, in the wine-growing regions of the Mosel and Rhine valleys, where the disease was named "Vergilbungskrankheit". Subsequent research confirmed that it was the same disease (Pierro et al., 2024). In the following years, BN was reported in numerous countries across the Euro-Mediterranean region, as well as in some parts of other continents, causing significant agricultural losses (Mori et al., 2024). In 2005, the first cases of BN disease in Bulgaria were recorded in the regions of Sliven, Targovishte, and Veliko Tarnovo, affecting the grapevine varieties Chardonnay and Merlot. According to observations in subsequent years, the number of affected areas gradually increased (Avramov & Mihaylov, 2019). In the Euro-Mediterranean region, '*Candidatus* Phytoplasma solani' is primarily transmitted between plants by the polyphagous vector *Hyalesthes obsoletus*, which is widely distributed across Europe and present in many European countries (Weintraub & Beanland, 2006; Johannesen et al., 2008; Sémétey et al., 2018; Avramov, 2022). The cicada is responsible for the widespread dissemination of stolbur during different periods in countries such as Israel, Spain, Italy, Greece, and others (CABI, 2021). Infected grapevines are not a significant source of inoculum, as the cicada prefers other food sources and only turns to grapevines as a last resort. This explains the relatively limited spread of BN and the possibility of infection even with a high population density of the vector in the vineyard area. However, the situation changes rapidly and dramatically when new vectors of phytoplasmas appear in the vineyards (Kosavac et al., 2019). Bois Noir in grapevines causes yellowing of the leaves in white varieties and reddening in red varieties. Characteristic symptoms also include downward curling of the leaves, stunted growth, as well as shriveling and drying of grape clusters and berries. Young plants may die after infection, while older ones usually recover (Pierro et al., 2024). The severity of the symptoms depends on the susceptibility of the specific variety (EFSA, 2016). The signs of the disease do not differ from those caused by grapevine flavescence dorée (EPPO, 2018). The main symptom associated with BN is the loss of yield due to berry deformation, leading to significant economic consequences, especially in sensitive varieties (EFSA, 2014; Mori et al., 2024). The article aims to raise awareness about the serious threat posed by phytoplasma diseases, such as FD and BN, to viticulture due to the significant economic losses they cause. It emphasizes the need for integrated control strategies, including early diagnosis, vector control, and the use of resistant grapevine varieties. Ultimately, the article seeks to draw the attention of specialists, producers, and regulatory authorities to the importance of prevention and control of phytoplasma diseases in order to safeguard Bulgarian viticulture from potential economic losses.

MATERIALS AND METHODS

The materials were received at the laboratory from various locations across Bulgaria. During the period 2022-2024 a total number of samples 667 were obtained, plant samples collected from

vineyards (466 samples), mother vineyards (171), rooting beds, rootstocks, scions, and cuttings (30) were analyzed. The number of samples for the year 2022 was 244, of which 161 samples were from vineyards and 83 samples were from mother vineyards. In 2023, a total of 230 samples were analyzed, including 180 samples from vineyards, 31 samples from mother vineyards, 12 samples from rooting beds, 6 samples from rootstocks, and 1 sample from scions. In 2024, the total number of samples analyzed was 192, of which 125 samples were from vineyards, 57 samples from mother vineyards, and 11 samples from cuttings. The diagnosis of phytoplasmas is routinely performed using PCR and can be divided into three phases: general extraction of DNA from symptomatic and asymptomatic tissue; PCR amplification of phytoplasma-specific DNA; and characterization of the amplified DNA through RFLP analysis or nested PCR with group-specific primers (Avramov, 2023).Total DNA was extracted from plant tissue (Doyle and Doyle, 1990) or a pre-determined individual in CTAB buffer following the protocol by Marzachi et al. (1998). Laboratory analysis was conducted using Nested PCR with two pairs of universal primers, P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1996) and U3/U5 (Lorenz et al., 1995), as well as specific primers f01/r01 (Lorenz et al., 1995), followed by RT-RFLP analysis using RsaI/AluI restriction enzymes for final identification and species classification (Lee et al., 1998).

RESULTS AND DISCUSSIONS

For the period 2022-2024, a total of 1,346 laboratory analyses were conducted on 667 plant samples, and phytoplasma infections were detected in 6 samples. No FD infection was found in any of the samples during this period. The results were presented in Table 1.

Table 1. Laboratory Analyses and Infections, 2022-2024

Year	Total samples	Total Analyses	BN infection	FD infection
2022	244	492	+	-
2023	230	462	+	-
2024	193	392	+	-
Total	667	1346	+	-

+ = positive result; - = negative result.

In 2022, one BN infection was detected in a vineyard in the Montana region and another BN infection in a vineyard in the Burgas region. In 2023, only one BN infection was recorded in a vineyard in the Ruse region. In 2024, two BN infections were identified in the Ruse region from mother vineyards (Table 2).

Table 2. Number of samples and infections of BN by year

Year	Mother vine yards	Infec tion	Vine yard	Infec tion	Root stocks	Infec tion	BN+
2022	83	-	161	2	-	-	2
2023	31	-	180	1	19	-	1
2024	57	2	125	1	11	-	3
Total	171	2	465	4	30	-	6

In 2024, there is an increase in cases in the Ruse region, which may indicate a localized risk of spread (Table 3).

The infected plant materials originate from three varieties - Chardonnay (4 positive results); Cabernet Sauvignon and Misket Hamburg and separated us following in a regions - Ruse 3 positive samples with confirmed detection of BN on Chardonnay and Misket Hamburg; Montana region with detection of BN on Cabernet Sauvignon and Chardonnay; Burgas region on Chardonnay (Table 3).

Table 3. FD and BN Infections by year and region

Region	Number of samples	Infection	
		FD	BN
Blagoevgrad	27	-	-
Burgas	65	-	1
Varna	74	-	-
Vidin	37	-	-
Vraca	44	-	-
Veliko Tarnovo	28	-	-
Dobrich	30	-	-
Lovech	12	-	-
Montana	51	-	2
Paazardjik	19	-	-
Pernik	1	-	-
Pleven	4	-	-
Plovdiv	43	-	-
Ruse	40	-	3
Sliven	49	-	-
Stara Zagora	21	-	-
Haskovo	16	-	-
Shumen	43	-	-
Yambol	49	-	-
Total for the country	677	6	

The recommended measures for the detected infections include adherence to quarantine measures, and uprooting of the vineyards is not recommended. Out of 1,332 laboratory analyses of 667 samples, only 6 positive cases were detected. No cases of Flavescence dorée (FD) were found, which is a positive indicator of the phytosanitary status of Bulgarian vineyards. Bois noir was first recorded in Bulgaria in 2005 in the regions of Sliven, Targovishte, and Veliko Tarnovo, affecting the wine grape varieties Chardonnay and Merlot. The result of the work confirms the date of previously detection, that the infection spread to additional viticultural areas, and had already been detected in all major wine-producing regions of the country (Avramov, 2023). Data from 2022 to 2024 indicate a stable level of BN spread, with small down level of changes, as 6 infections were found among 667 analyzed samples. The distribution of infections by grape variety shows that wine varieties are the most affected (1.08%), followed by local and table grape varieties (0.58%) (Table 4).

Table 4. Infected varieties by BN for 2022-2024

Region	Infected varieties by BN	
Ruse	Chardonnay (2)	Misket Hamburg
Montana	Chardonnay	Cabernet Sauvignon
Burgas	Chardonnay	-

This confirms the observed higher susceptibility of wine grape varieties to phytoplasma infections. The Chardonnay variety appears to be particularly vulnerable, with BN detected in 4 samples from various regions of the country. Although BN was identified in 1 analyzed grapevine varieties, the overall infection rate remains relatively low, possibly due to the absence of favorable factors for its spread, such as the origin of planting material, varietal specificity, vineyard age, agricultural practices, and pest management systems. This suggests that some varieties are more susceptible to BN infection, while others are more resistant (Avramov, 2023). During the most recent analyzed period 2022-2024, a significant decrease in BN cases has been observed compared to previous years. Although the overall prevalence of BN remains low, an increase in cases was noted in the Ruse region in 2024. This may indicate a localized risk of infection spread in this area. The decreased frequency of BN infections in recent years may be attributed to improved phytosanitary control, the effective application of integrated pest management strategies, and strict monitoring of planting material. However, the localized increase in cases in Ruse requires attention and additional preventive measures. Key risk factors for BN spread are: presence of vectors (*Hyalesthes obsoletus*) - although grapevines are not the primary host of this vector, an increase in its population in a given area can raise the risk of infection transmission; use of infected planting material - despite strict regulations, the possibility of BN spreading through infected cuttings remains. Recommended measures to limit BN spread are**:** Enhanced monitoring and surveillance - in regions with confirmed cases (Ruse), more active diagnostics are necessary to prevent further spread; Vector control - integrated pest managment strategies to control *Hyalesthes obsoletus*, including biological control and agricultural practices, can reduce the risk of transmission; Strict control of planting material - ensuring healthy planting material through in-depth laboratory testing before replanting and Vineyard management-improving agricultural practices, including sanitary pruning and optimizing plant protection systems.

CONCLUSIONS

In conclusion the data from the 2022-2024 period indicate a significant decrease in BN cases in Bulgaria compared to previous years. The registered increase in cases in the Ruse region in 2024 highlights the need for intensified monitoring and the application of effective preventive measures. The absence of Flavescence dorée cases is a positive indicator of the health of Bulgarian vineyards, but continued systematic control and adherence to phytosanitary measures remain crucial for limiting the spread of phytoplasma infections in the country. The present study provides up-to-date information on the status of phytoplasma diseases in grapevines in Bulgaria for the period 2022-2024, with a focus on FD and BN. The data highlight the need for continued strict

monitoring and the implementation of integrated control strategies, including early diagnosis, vector management (primarily *Hyalesthes obsoletus*), and the use of resistant grapevine varieties.

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AGROBIOLOGICAL CHARACTERISTICS OF SOME AUTOCHTHONOUS TABLE GRAPE VARIETIES UNDER THERMOHYDRIC STRESS IN SOUTHERN ROMANIA

Georgeta Mihaela BUCUR[1], Elena DELIAN[1], Marinela Vicuța STROE[1], Roxana Mihaela FILIMON[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Development Station for Viticulture and Winemaking Iaşi, 48 Mihail Sadoveanu Alley, 700490, Iaşi, Romania

Corresponding author`s emails: mihaela_g_savu@yahoo.com, roxanacotovanu@yahoo.com

Abstract

In this study, we followed the physiological characteristics of three local table grape varieties, under conditions of thermal and hydric stress, which have an essential role in their transpiration and implicitly drought resistance. The varieties 'Augusta', 'Victoria' and 'Xenia' were studied, in the phenophases of flowering, berry growth, veraison and ripening. The content of chlorophyll and carotenoid pigments was generally sub-unitary, compared to the values reported for grapevines (1.05-1.58 mg 100 g^{-1} FW). Regarding the number of stomata, the most were determined in the 'Augusta' variety (123 stomata/mm^2), followed by 'Xenia' (98 stomata/mm^2) and 'Victoria' (88 stomata/mm^2). The photosynthesis rate recorded maximum values in the flowering phenophase in the 'Augusta' (16.14 µmols CO_2 m^{-2}s^{-1}) and 'Xenia' varieties (12.37 µmols CO_2 m^{-2}s^{-1}), possibly also due to a high requirement for photoassimilates. In the 'Victoria' variety, the highest value was reached at veraison (11.19 µmols CO_2 m^{-2}s^{-1}). As a result, the recorded productions were diminished, without affecting the quality of the grapes.

Key words*: table grape varieties, physiology, thermohydric stress.*

INTRODUCTION

The complexity of the Romanian geographic landscape creates favorable ecoclimates for the cultivation of wine grape varieties, but also of table varieties - more demanding in terms of heliothermal and hydric conditions. Moreover, the current climatic warming favors this production direction.

In the past, the most favorable areas for table grapes varieties were those situated in the southern half of the country (Oşlobeanu et al., 1980; 1991). In the latest decades, as a result of climatic warming, the cultivation of these varieties, with different maturation periods, has also developed in the northern part of the country (Rotaru et al., 2011; Filimon et al., 2016).

Grapes, apart from wine production, are highly appreciated for fresh consumption; in addition to a significant nutritional value, table grapes also have therapeutic actions, with beneficial effects on the human body.

The quality of table grapes must be appreciated from the point of view of both the consumer, the producer and the trader (Bucur, 2024).

The consumer is interested in the organoleptic quality of grapes. It is based on the overall consideration of olfactory (aroma), gustatory (flavor, acidity, sugar content), tactile (pulp consistency), visual factors (color, size, freshness etc.).

For the producer, the cultivation of table grapes is a profitable activity. The producer is interested in the maximum value of the goods he puts on the market, so the concept of quality must take into account both the consumer and the trader. Using valuable varieties, well placed in the field and properly cared for, the productions obtained are much higher compared to wine varieties. Production costs are recovered in a shorter time for table grapes than for wine grapes, therefore the rotation of financial means takes place faster.

For the trader, quality is assessed by homogeneity, the size of the grapes and berries, the external appearance, resistance to transportation and storage etc.

In the last 50 years, as a result of research work carried out in our country, numerous new table grape varieties have been approved and introduced for propagation, as well as valuable clones, appreciated not only in the country but also abroad (Indreaș & Vișan, 2000). These include 'Augusta', 'Victoria' and 'Xenia', which present a series of characteristics appreciated by the modern consumer, producer and trader.

The present study was carried out in the ampelographic collection of the Faculty of Horticulture in Bucharest (a region located in the Romanian Plain, characterized by a fairly high effective temperature), to examine the agrobiological characteristics of some Romanian table grape varieties, in an attempt to determine their behavior and adaptation in semi-arid climatic conditions. Thus, we can rationally choose and apply certain technological links (pruning, green operations, solar protection, irrigation, fertilization etc.), compatible with high quality (Dobrei et al., 2023; Stănuș et al., 2024).

Climate change (global warming, different distribution of precipitation during the growing season, with increased water stress during grape ripening, greater frequency of extreme weather events etc.) produces physiological and biochemical changes in the growth and fruiting of the vine, influencing grape production and especially its quality. Heat waves cause an acceleration of the development of phenophases, especially grape ripening, with large accumulations of sugars, with a sharp degradation of acidity, increased pH values and the development of atypical aromatic compounds.

MATERIALS AND METHODS

The biological material used and the study site

On carried out the study within the framework of the Faculty of Horticulture, located in the southern part of Romania, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest (N Lat.: 44° 47' 07"; E Long.: 26° 07' 28"; alt. 87 m).

The grapevine cultivars analized in this study in year 2024, all of them for table, were with green yellow berry skin color ('Augusta', 'Victoria' and 'Xenia'); the biological material subjected to the study is presented in Figure 1.



'Augusta'
Genitors - *Italia x Regina viilor*
VIVC no - 14781



'Victoria'
Genitors - *Cardinal x Afuz Ali*
VIVC no - 13031



'Xenia'
Genitors - *Bicane x Muscat de Hamburg*
VIVC no - 13274

Figure 1. Grape varieties studied

'Augusta' - table grape variety obtained at Nicolae Bălcescu Agronomic Institute Bucharest, by Neagu Marin and Georgescu Mihai, homologated in 1984. The variety complements the assortment of early maturing table grape varieties, being appreciated for its earliness, production, taste qualities, berry size, low number of seeds etc. (Georgescu et al., 1986).

'Victoria' - table grape variety obtained at the Drăgășani Viticulture and Winemaking Research Station, by Lepădatu Victoria and Condei Gheorghe, homologated in 1978. Is one of the most valuable Romanian table grape varieties, being cultivated in other wine-

growing countries such as Italy, France, Spain, Portugal, Greece, China, South Africa, Australia etc. (Glăman et al., 2018).

'Xenia' - table grape variety obtained at the Greaca Viticulture and Winemaking Research Station, by Gorodea Grigore and Lumînare Zamfirița, homologated in 1983. The variety completes the varietal conveyor of late table varieties, for fresh consumption and winter storage.

Varieties are planted at a spaced by 2.2 m (inter-row) and 1.2 m (intra-row), with a density of 3787 plant·ha^{-1}, rows direction N-S, in a trellis system in a Guyot pruning type semi-tall.

No irrigation was performed in the vineyard during the vegetation season when the determinations were made and phytosanitary treatments against to control diseases and pests have been applied in accordance with local standard practice. Soil management provides for natural ground cover.

Climatic conditions

For this study, there were used weather data recorded at Bucharest-Baneasa meteorological station for the year 2024, as compared to multi-annual values (1991-2020). Monthly average temperatures were used to calculate a set of bioclimatic indices commonly used in viticulture.

Parameters determined

Analysis of the phenological spectrum

The recording of the development of the vegetation phenophases (budburst, flowering, veraison, harvesting maturity) will be based on the specifications made in the OIV Descriptor List - second edition (2009), according to BBCH (**B**ioligische **B**undesansalt und **Ch**emische Industrie). Data were recorded at which 50% of buds, flowers, grapes reached the respective phenological stages (BBCH 008 - budburst; BBCH 605 - flowering; BBCH 801 - veraison and BBCH 809 - berries ripe for harvest).

Quantification of Photosynthetic Pigments

The determinations on the variation of chlorophyll and carotenoids content pigments were carried out at the beginning of flowering, berry growth, veraison and in the phenophase of grape ripening.

Analytical determinations were performed using a UV-vis spectrophotometer, at wavelengths: 663, 646 and 470 nm, using as a solvent acetone 80% (Lichtenthaler, 1987). Results were expressed as mg 100 g^{-1} FW fresh mass.

Determination of leaf moisture

In parallel with the determination of the content of photosynthetic pigments in the leaves, the moisture content (%) of the leaves was also determined, by drying for minimum of 4 hours in an oven, at a temperature of 105±2C (until constant mass).

Stomatal density measurement

Stomata density (SD) in leaves has been analysed on mature healthy, in July 2024, during the berries growth phase (the size of a pea), by the method of stomatal impression described everywhere in specialized works.

A thin layer of nail polish was applied on the abaxial leaf side, on an area of about 2 cm^2. After solvent evaporation (about 20 minutes later), the transparent stomatal impression of the leaf epidermis was taken with the help of a sheet of transparent shells and placed on a microscopic slide, which was labelled with the name of the sample. The observations were made with the optical microscope. SD was counted and expressed as number per mm^2.

Leaves gas exchange parameters

Photosynthesis rate (A - µmols CO_2 m^{-2}s^{-1}) and transpiration rate (E - mmol H_2O m^{-2}s^{-1}) have been measured *in-situ*, for leaves situated at the middle third of the shoot (nodes 4-7), in the morning, between 09:00 and 11:00, in the flowering phenophase, berries growth (pea size) and at the beginning of the ripening, using the portable infrared gas analyser (LCPro-SD-ADC BioScientific Ltd, Hoddesdon, UK).

Quantitative and qualitative parameters at harvesting

At harvesting determinations were made on quantitative (grape weight - grams, weight of 100 berries – grams) and qualitative parameters (sugar content - ºBrix, titratable acidity - g/L tartaric acid, pH, ºBrix/acidity ratio, polyphenolic potential). Sugar concentration in grapes was measured by using an Atago digital refractometer. The results were expresed in ºBrix. Titratable acidity was determined by titrating with 0.1 N NaOH using an Pellet digital biurette, and expresed as g/L tartaric acid; while polyphenolic potential of the

harvest were assessed according to ITV method.

Statistical analysis

Data were processed using Microsoft Excel (version 2010) and are shown as average values ± Standard Deviation (SD).

The analysis of variance (ANOVA) was performed. Then, the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software was carried out to determine where there were statistically significant differences. Statistically significant differences have been considered at the value of $p \leq 0.05$.

Different lowercase letters denote significant differences when comparing cultivars within the same phenophase. Different uppercase letters denote significant differences when comparing a cultivar, taking into account the phenophase in which the respective indicator was determined.

RESULTS AND DISCUSSIONS

Climatic conditions

In the year 2024, higher temperatures were recorded, compared to the multiannual average, both in terms of average temperature and annual maximum temperature (Table 1). The growing season in 2024 was drier, and summer rainfall (VI-VIII) was lower than usual. Heat waves, assessed based on the number of days with maximum temperatures above 30ºC and 35ºC (the heatwave threshold), have experienced an upward trend in the last two years in the southern part of the country and beyond. Due to intense solar radiation and extreme temperature values during the grape ripening period, sunburn was recorded in grapes on the western side of the rows oriented in the N-S direction.

The hydrothermal coefficient recorded on a low value (0.64), which indicates a dry area, where the cultivation of table grape varieties becomes economical only under irrigated conditions, with moderate irrigation also necessary for wine varieties. The Huglin Index (HI) has increased to very high values, both in terms of the multiannual average (HI = 3163) and in the year 2024 (HI = 4138). The increase in the Huglin Index indicates the evolution of the local climate towards a very warm climate (HI+3), which is now typical of regions such as Malaga (Spain) or Marsalla (Italy) (Irimia & Patriche, 2019). The values of the Winkler index and Cool night index were also higher as compared to the multiannual average. These climatic conditions have led to changes in the development of the phenophases, the grapes yield and its quality.

Table 1. The main climatic indicators of the year 2024 compared to the multiannual average (1991-2020)

Climatic indicator	Multiannual average (1991-2020)	2024
Average annual temperature, ºC	10.98	14.70
Average temperature in the growing season, ºC (IV-X)	17.46	20.62
Average temperature in summer, ºC (VI-VIII)	22.15	26.30
Average annual minimum temperature, ºC	5.24	7.70
Average annual maximum temperature, ºC	17.6	21.10
Number of hot days (T_{max} > 30°C)	46	52
Number of very hot days (T_{max} > 35°C)	10	38
Annual total precipitation, mm	633	583
Total precipitation in the growing season (IV-X), mm	430	339
Total precipitation in summer (VI-VIII), mm	193	180
Hydrothermal coefficient (HC)	1.1	0.75
Huglin index (HI, Huglin, 1978)	3163	4138
Winkler index (WI, Winkler, 1974)	1762	2273
De Martonne Aridity Index (IDM, year)	30.3	23.6
Cool night index (CI, Tonietto and Carbonneau, 2004)	10.64	13.5

Analysis of the phenological spectrum

In a rational viticulture, in search of an optimal organoleptic quality, the climatic factors involved are more complex; and the climatic parameters taken into account are, in general, temperature, insolation and precipitation. In order to characterize the genetic resources in grapevine, it is important to record the main phenophases correlated with these parameters, especially with the thermal ones. Knowing the moment when these phenophases occur in different varieties is necessary not only for the understanding of the vineyard physiology, the application of pesticides, fertilizers, irrigation at the right time, the correct execution of the green aplication, but also for their influence on yield and quality attributes (Bucur & Dejeu, 2018).

Table 2 shows the average data of the four main phenological stages (budburst, flowering, veraison and berries ripe for harvest); climatic factors from the year 2024 directly influencing the onset and their development. Thus, the beginning of the phenophases varied depending on the average daily air temperatures, the useful heat balance (UHB) being calculated.

Budburst occurred 10-20 days earlier in year 2024 (April 5-10) for 'Augusta' and 'Victoria' varieties, respectively with 10-15 days earlier for 'Xenia' variety, compared to literature data (April 15-25) (Bucur & Dejeu, 2018; Glăman et al., 2018).

The flowering took place earlier in the 'Augusta' and 'Xenia' varieties (May 25), and with about 5 days later to the variety 'Victoria' (May 31).

The beginning of berry ripening (veraison) took place on the 22 July in the 'Augusta' and 'Victoria' varieties, and later in 'Xenia' (August 5-7).

Grape ripening took place on August 5 for the 'Augusta' variety, followed by the 'Victoria' variety. The 'Victoria', appreciated for its early ripening and commercial value of the grapes, reached organoleptic maturity on August 12, knowing that this variety ripens starting from the 2nd-3rd decade of August in the wine-growing areas of Romania (Lepădatu & Condei, 1986; Olteanu et al., 2002; Stroe, 2016; 2021).

This variety makes good use of its early full ripening, having an advance of at least one week compared to other cultivation areas in our country, in the ecoclimatic conditions of southwestern Romania (Plenița vineyard) (Cichi et al., 2023).

The greatest variation in grape ripening was recorded for 'Xenia'. Under the temperate-continental climate of Romania, grape ripening for this cultivar, takes place between September 15-20, and in the south of the country somewhat earlier, on August 26-28.

It should be noted that under the climatic conditions of the year 2024, 'Xenia' reached organoleptic maturity on August 12, 15-28 days earlier than other growing areas in the country.

Table 2. The phenological spectrum of the studied varieties (ampelographic collection of USAMV of Bucharest, 2024)

Variety	Budburst (50%)		Flowering (50%)		Veraison (50%)		Harvest	
	Date	UHB* (°C)	Date	UHB* (°C)	Date	UHB* (°C)	Date	UHB* (°C)
'Augusta'	05.04	18.5	25.05	239.5	22.07	866	05.08	224
'Victoria'	10.04	41.0	31.05	260.5	22.07	813	12.08	305
'Xenia'	05.04	18.5	25.05	239.5	05.08	1074	12.08	133

*useful heat balance

Quantification of photosynthetic pigments and determination of leaf moisture

Photosynthetic pigments make up the receptor antennae and reaction centers of the two photosynthetic systems and participate in the process of photosynthesis. The chlorophyll a/b ratio is maximal at the beginning of the vegetation period, reaching up to 3/1 and decreases during grape ripening, while the chlorophyll/carotenoid ratio can register values of 4/1 (Filimon et al., 2016).

Assimilating pigments have an important role in plant growth, in the oxidation-reduction and fruiting processes, the variation of these parameters being an indicator of stress or damage to the leaf apparatus (Ghiur et al., 2022).

As can be seen in Table 3, chlorophyll *a* reached the highest value in the flowering

phenophase in the 'Augusta' variety (101.0 mg 100 g^{-1} FW), and in the phenophases of berry growth, veraison and harvest it recorded the highest values in the 'Xenia' variety (97.91 mg 100 g^{-1} FW, 115.25 mg 100 g^{-1} FW, and 116.06 mg 100 g^{-1} FW, respectively).

Chlorophyll *b* recorded high values in the flowering and berry growth phenophases in the 'Augusta' variety (60.56 mg 100 g^{-1} FW, respectively 59.30 mg 100 g^{-1} FW), and in the veraison and ripening phenophases the highest value was taken over by the 'Xenia' variety (76.22 mg 100 g^{-1} FW, respectively 79.55 mg 100 g^{-1} FW).

Carotenoids had the highest values in the flowering (26.34 mg 100 g^{-1} FW), veraison (26.51 mg 100 g^{-1} FW) and ripening (25.83 mg 100 g^{-1} FW) phenophases in the 'Victoria' variety, and in the berry growth phenophase in the 'Xenia' variety (26.35 mg 100 g^{-1} FW).

After determining the content of photosynthetic pigments, *the humidity of the leaves* of the studied grape varieties was also determined. According to the data in Table 3, the humidity recorded the highest values at flowering (in the third decade of May), for the varieties 'Augusta' (73.11%) and 'Xenia' (72.43%), followed by a gradual decrease in July and August. For the variety 'Victoria' the humidity recorded the highest value during the period of berry growth (73.03%).

Table 3. Assimilatory pigments contents and leaf moisture of the studied varieties

Variety	Chl *a* (mg 100 g^{-1} FW)	Chl *b* (mg 100 g^{-1} FW)	Carotenoids (mg 100 g^{-1} FW)	Moisture content of the leaf (%)
Flowering				
'Augusta'	101.00 ± 0.86aB	60.56 ± 8.83aAB	25.82 ± 1.58aA	73.11 ± 3.11A
'Victoria'	89.85 ± 8.71bB	34.26 ± 10.39bB	26.34 ± 2.01aA	71.95 ± 2.24A
'Xenia'	82.32 ± 1.79bC	28.30 ± 4.82bC	23.11 ± 2.11aAB	72.43 ± 0.41A
Berry growth				
'Augusta'	97.59 ± 0.61aB	59.30 ± 5.17aAB	23.37 ± 0.53cB	69.29 ± 1.55B
'Victoria'	97.62 ± 1.95aAB	52.05 ± 6.84aA	25.35 ± 0.47bA	73.03 ± 0.85A
'Xenia'	97.91 ± 2.74aB	48.28 ± 3.55aB	26.35 ± 0.09aA	70.43 ± 2.58A
Veraison				
'Augusta'	100.46 ± 1.63bB	50.57 ± 5.45bB	26.20 ± 1.27aA	65.88 ± 3.09BC
'Victoria'	102.83 ± 1.86bA	55.60 ± 5.14bA	26.51 ± 0.69aA	58.70 ± 14.58A
'Xenia'	115.25 ± 4.54aA	76.22 ± 10.15aA	21.99 ± 3.22bBC	63.48 ± 0.69B
Grape ripening				
'Augusta'	108.92 ± 7.24abA	66.25 ± 6.33abA	20.59 ± 0.89aC	64.06 ± 1.45C
'Victoria'	101.86 ± 2.90bA	53.09 ± 7.90bA	25.83 ± 1.30aA	66.83 ± 0.73A
'Xenia'	116.06 ± 3.68aA	79.55 ± 7.05aA	18.82 ± 0.98aC	59.90 ± 1.01C

Legend: Data are shown as mean value ± Standard Deviation (N = 3). The significant differences ($P < 0.05$) between variants are indicated by different letters in the row: different lowercase letters denote significant differences when comparing cultivars within the same phenophase; different uppercase letters denote significant differences when comparing a cultivar, taking into account the phenophase in which the respective indicator was determined.

Stomatal density measurement

Stomata are the main route of water removal from plants, through the process of transpiration, but also the place where carbon dioxide enters, which is a raw material in the photosynthesis process. These tissues called stomata are found on epidermal surfaces of all plant organs which contact with air.

From the data presented in Table 4, it can be seen that there are no significant differences in the number of stomata in the three varieties studied.

Table 4. Determination of the number of stomata

Variety	Number of stomata/mm^2
'Augusta'	123.33 ± 8.50a
'Victoria'	97.67 ± 32.02a
'Xenia'	88.00 ± 1.41a

In grapevines, the leaf surface varies depending on the grape variety, the ecological conditions in the growing area, fertilizers used, the irrigation regime, and grapevines have quite large leaf surfaces (Odabasioglu & Gursoz, 2019).

Leaves gas exchange parameters

▪ *Analyzing the intensity of the photosynthesis process* during the different phenophases (Table 5) it is found that the intensity of this process varied between 7.92 and 16.14 µmol CO_2 $m^{-2}s^{-1}$ in the flowering phase (May 31), between 5.82 and 8.19 µmol CO_2 $m^{-2}s^{-1}$ in the berry growth phenophase (June 26) and between 3.27 and 11.19 µmol CO_2 $m^{-2}s^{-1}$ in the ripening phase (July 22, August 05).

▪ *Analyzing the intensity of the transpiration process* during different phenophases (Table 5), it was found that the intensity of this process varied between 4.77 and 9.42 mmol H_2O $m^{-2}s^{-1}$ in the flowering phase (May 31), between 5.04 and 9.42 mmol H_2O $m^{-2}s^{-1}$ in the berry growth phenophase (June 26) and between 1.68 and 9.39 mmol H_2O $m^{-2}s^{-1}$ in the ripening phase (July 22, August 05).

It can be noted that in the flowering phenophase there are significant differences between the three varieties, while in the berry growth phenophase the differences recorded are statistically insignificant. The beginning of berry ripening (veraison), statistically significantly lower values were recorded for 'Xenia'.

For each variety studied physiological parameters evolved differently.

For the variety 'Augusta' the intensity of photosynthesis peaked in the flowering phenophase, possibly also due to a high requirement for photoassimilates, then showed a marked decrease in the berry growth phenophase and then the process showed a slight increase. *The transpiration intensity* peaked at the berry growth phenophase (5.04 mmol H_2O $m^{-2}s^{-1}$).

In 'Victoria' we note that the intensity of photosynthesis increased progressively from flowering to beginning of berry ripening (veraison), when it reached its maximum value (11.19 µmol CO_2 $m^{-2}s^{-1}$). *Transpiration intensity* was high in all three phenotypes, with no marked differences between phenotypes.

In 'Xenia', the maximum values of photosynthesis rate were recorded at flowering (12.37 µmol CO_2 $m^{-2}s^{-1}$), when water use efficiency had the highest values, then gradually decreased, reaching 3.27 µmol CO_2 $m^{-2}s^{-1}$ at the fallow stage. *The intensity of the transpiration process* was also highest at flowering (5.72 mmol H_2O $m^{-2}s^{-1}$), and then gradually decreased, reaching its lowest value in the veraison phenophase (1.68 mmol H_2O $m^{-2}s^{-1}$).

Table 5. The main physiological parameters recorded of the studied varieties, Bucharest 2024

Variety	Flowering	Berry growth	Veraison
A - Net photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)			
'Augusta'	16.14 ± 2.71aA	5.82 ± 0.59aB	8.73 ± 2.19aB
'Victoria'	7.92 ± 0.23bB	8.19 ± 1.80aB	11.19 ± 1.72aA
'Xenia'	12.37 ± 1.77cA	7.35 ± 0.86aB	3.27 ± 1.03bC
E - Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)			
'Augusta'	4.77 ± 1.53bA	5.04 ± 1.51bA	3.64 ± 1.73bA
'Victoria'	9.42 ± 3.27aA	9.42 ± 0.57aA	9.39 ± 0.28aA
'Xenia'	5.72 ± 0.60abA	4.16 ± 1.46bA	1.68 ± 0.68bB

Legend: Data are shown as mean value ± Standard Deviation (N = 3). The significant differences ($P < 0.05$) between variants are indicated by different letters in the row: different lowercase letters denote significant differences when comparing cultivars within the same phenophase; different uppercase letters denote significant differences when comparing a cultivar, taking into account the phenophase in which the respective indicator was determined.

Our obtained data are in a great measure consistent with those of Williams & Biscay (1991), who found that the intensity of the photosynthetic process ranged from 13.8 µmol µmol CO_2 $m^{-2}s^{-1}$ in August to 7.5 µmol µmol CO_2 $m^{-2}s^{-1}$ in September. Also, Botos-Balo et al. (1992), found that leaves of *Vitis vinifera* plants have an average net photosynthesis intensity of 11.6 µmol CO_2 $m^{-2}s^{-1}$, and Lasko et al. (2000), state that it varies between 16 and 18 µmol CO_2 $m^{-2}s^{-1}$. Determinations carried out by Burzo et al. (2001) on 28 grapevine varieties revealed that the intensity of photosynthesis ranged from 5.26 µmol CO_2 $m^{-2}s^{-1}$ in the variety 'Kiș Miș white', 10.44 µmol CO_2 $m^{-2}s^{-1}$ in the variety 'Victoria' and 12.08 µmol CO_2

$m^{-2}s^{-1}$ in the variety 'Rkatiteli', under similar temperature conditions (34-36ºC) and light intensity of 1.820-1.870 moli/m^2/s.
Villalobos-Gonzalez et al. (2022), found during the vegetation period, photosynthesis intensity values ranging between 4.68 and 12.02 μmol CO_2 $m^{-2}s^{-1}$ under the conditions of a year with a rainfall deficit, in the varieties 'Chardonnay', 'Carménère', 'Sauvignon Blanc' and 'Cabernet Sauvignon', which are stomatal sensitive.
The determinations carried out by Bucur et al. (2024) indicated that the intensity of this process varies between 3.88 and 13.3 μmol CO_2 $m^{-2}s^{-1}$, in the case of applying treatments with kaolin and zeolite in concentrations of 3 and 5% to the 'Fetească regală' variety, intended to mitigate the effects of summer thermohydric stress.

Quantitative and qualitative parameters at harvesting

Table 6 presents the main quantitative and qualitative parameters at harvesting time of the studied varieties. The average grape weight data represent the average of three replicates, each one for three representative grapes, according to the methodology established under COST Action FA 1003.
The quality of the grapes, indicated by the average weight of the berries, the sugar content, titratable acidity and ºBrix/acid ratio (proportion), reflected both the specific genetic character of each individual genotype and the influence of climatic factors on these elements.
The analyzed varieties were characterized by medium-large grapes, with an average weight between 544 g ('Victoria'), 315 g ('Augusta') and 253 g ('Xenia') (Table 6), the current value of 'Xenia' cultivar being close to that reported by Irimia et al. (2009).
The weight of 100 berries, was specific to each genotype, ranging from 380 g for the grapes of 'Xenia' variety, 564 g for the 'Augusta' to 681 g for 'Victoria'. Table grape varieties are usually harvested at a lower sugar content, compared to wine varieties. According to The International Organisation of Vine and Wine (OIV), resolution VITI 1/2008, grapes with a Brix degree equal to or above 16 shall be considered riped (OIV, 2008). For white/pink table grape varieties, grapes with a Brix degree under 16 must have a minimum sugar (expressed in g/L) / acid (expressed in g/L of tartaric acid) of 20/1 proportion, in order to be considered riped.
In the EU, the minimum refractometric index shall be above of 12 ºBrix for the varieties 'Alphonse Lavallée', 'Cardinal' and 'Victoria', 13 ºBrix for all other seeded varieties, and 14 ºBrix for all seedless varieties. In adition, all varieties must have satisfactory sugar/acidity proportion levels.
In the climatic conditions of the year 2024, the studied varieties showed average to high sugar concentrations (16.9 ºBrix 'Victoria' variety, 19.3 ºBrix 'Xenia' and 19.6 ºBrix 'Augusta'). 'Xenia' variety grapes showed the lowest total acidity of the must (3.68 $g.L^{-1}$ tartaric acid), while 'Victoria' variety presented a low sugar-acidity ratio, due to higher acidity of grapes.
ºBrix/acid ratio can be used as predictor for the table grapes consummer acceptability, better for the sensory attribute as compared to the ºBrix or acidity values (Jayasena & Cameron, 2008; Irimia, 2012).
The total polyphenolic index determined by the ITV method (extraction of crushed berries with 0.1% HCl and 95% ethanol) showed values between 14.48 and 46.26, the highest value being obtained for the 'Xenia' variety grapes.

Table 6. Grapes quantitative and qualitative parameters at harvesting time of the studied varieties, Bucharest 2024

Variety	Grape weight (g)	Weight of 100 berries (g)	Sugar content (ºBrix)	Titratable acidity ($g.L^{-1}$ tartaric acid)	pH	ºBrix / acidity ratio	The polyphenolic potential of grapes	
							DO 280 nm	Polyphenolic index
'Augusta'	315	564	19.6	3.90	2.7	50.2	0.073	21.918
'Victoria'	544	681	16.9	3.86	2.9	43.7	0.048	14.484
'Xenia'	253	380	19.3	3.68	3.9	52.4	0.154	46.259

CONCLUSIONS

Analyzing climate data from 2024, compared to the multi-year average (1991-2020), climate warming is evident, both in terms of average temperature, precipitation, but especially heat waves felt by the number of days with maximum temperatures above 30°C, respectively 35°C, but also by the time when these temperatures occurred (in April there were days with temperatures above 30°C). Thus, the budburst phenophase started very early (05.04.2024 - 10.04.2024) in all the varieties studied. The other phenophases were also 7-10 days early. It should be noted that under the climatic conditions of the year 2024, 'Xenia' reached organoleptic maturity on August 12, 15-28 days earlier than other growing areas in the country.

The study of new varieties revealed a great phenological plasticity, the development of phenophases being dependent not only on the genetic factor, but also on the environmental conditions, mainly by the evolution of the temperatures that are constantly increasing. From a physiological point of view, considering the specific characteristics of the three varieties and the variability of the results obtained from this study, it is not possible to draw a clear conclusion on the superiority of one or the other in terms of photosynthetic yield and even less so in terms of transpiration rate.

The sugar content of the three table varieties ('Augusta', 'Victoria' and 'Xenia') was high, but together with the lower acidity resulted in a balanced gluco-acidimetric index.

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SECONDARY METABOLITES ACCUMULATION IN LONG-TERM CALLUS CULTURES OF *VITIS VINIFERA* CV. NEGRU VÂRTOS UNDER DIFFERENT STRESS CONDITIONS

Rodica CATANĂ[1], Raluca MIHAI[2], Larisa FLORESCU[1], Monica MITOI[1]

[1]Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, District 6, 060031, Bucharest, Romania
[2]CIAM, Department of Life Science and Agriculture, Universidad de Las Fuerzas Armadas - ESPE, Av. General Ruminahui s/n y, Sangolqui, 171103, Ecuador

Corresponding author email: rodica.catana@ibiol.ro

Abstract

Negru Vârtos is a variety of Vitis vinifera cultivated since the Dacian period. It is one of the most appreciated varieties in Romania, giving it a balanced and intensely colored wine. Our research aimed to increase the production of secondary metabolites in a long-term V. vinifera cv. Negru Vârtos callus culture obtained many years ago. Seven different abiotic factors represented by different media composition, salt addition, and UV or low-temperature exposure were tested to achieve this goal. The phenolic, flavonoid, anthocyanidin, and proanthocyanidin content was determined. Also, antioxidant activity was estimated by the α-diphenyl-α-picrylhydrazyl free radical-scavenging method (DPPH). Although all the tested variants showed better results concerning the callus morphometrical parameters as callus survival percentage, callus growth index, or callus moisture, only the variants treated with a low temperature of 4°C for 24 h revealed an increase of flavonoids, proanthocyanidins, and anthocyanidins content. Also, the production of anthocyanin was slightly stimulated in the R2 variant.

Key words*: abiotic stress, long-term callus, metabolites with biological activity, phenolic content, Vitis.*

INTRODUCTION

Vitis vinifera, the common grape vine, is an important crop plant that produces a variety of secondary metabolites with beneficial properties, such as resveratrol, anthocyanins, and flavonoids, compounds with recognized antioxidant, anti-inflammatory, and anticancer activities (Ali et al., 2024; Radulescu et al., 2020).

Negru vârtos (synonyms Gordan Negru, Corb, Neagră Vârtoasă, Negru Bătut, Negru Tare, Corb Negru) named because of the black colour of the grape skin, is one of the *V. vinifera* varieties of cultivated in Romania before the invasion of *Phylloxera* (Constantinesu, 1958); characterized by a long growing season with high production potential, with late ripening (https://grapevinevariety.com/details/show/4892), with unknown ancestry, used as a crossing partner for the Negru de Drăgășani and Novac varieties. No stocks have been reported since 2016 (https://glossary.wein.plus/negru-virtos).

Plant tissue culture offers alternatives for investigating metabolic pathways and factors influencing the biosynthesis of natural products of interest, in a controlled environment, independent of seasonal and geographical conditions. One of the possibilities for the production of valuable metabolites *in vitro* is based on callus culture (Ozyigit et al., 2023; Grąbkowska et al., 2016). Callus culture (an undifferentiated plant cell mass) is a valuable tool for studying and producing valuable compounds, including secondary metabolites (Ozyigit et al., 2023). Secondary metabolites represent compounds produced by plants, non-essential for growth, development, or reproduction, but with great benefits in adapting to environmental conditions (Seker & Erdogan, 2023). Different stress conditions can trigger the production of secondary metabolites in plants (Ramakrishna et al., 2011).

Over time, the long-term callus cultures become increasingly homogeneous (Fehér, 2019). In this context, the long-term callus cultures of *V. vinifera* represent a valuable source of natural antioxidants with potential applications in various industries (Sharafan et al., 2023).

Proanthocyanidins are a type of polyphenol, found in high concentrations in grapes (seeds and skin) with antioxidant properties with potential health benefits. Anthocyanins, a very attractive class of natural compounds, have different applications as colorants (food industry), nutraceuticals and therapeutic compounds (pharmacy), UV protectors, antioxidants, and anti-cancer compounds (cosmetics industry). Its biosynthesis is strongly induced by various stress factors (Hatier & Gould, 2009).

Our study aimed to evaluate the accumulation of the secondary metabolite (polyphenols, proanthocyanins, anthocyanidins, and flavonoids) in long-term callus cultures of *V. vinifera* cv. Negru Vârtos under different stress conditions.

MATERIALS AND METHODS

Plant material. The plant material used for our experiments was represented by *Vitis vinifera* long-term callus culture maintained for more than 40 years *in vitro* conditions. This long-term callus culture of *V. vinifera* cv Negru Vârtos was initiated in 1980 from leaf fragments from *in vitro* plantlets cultivated on a basal Gamborg (1968) medium supplemented with 0.1 mg/l NAA (α- naphthalene acetic acid), 0.2 mg/l kinetin, 2 g/l casein hydrolysate, 30 g/l sucrose, 8 g/l Plant Agar (Duchefa Biochemie, The Netherlands) (Brezeanu et al., 1980). The callusculture was subcultivated, over the years, on the MS (Murashige & Skoog, 1962) (Duchefa Biochemie, The Netherlands) media variant supplemented with the same additives previously mentioned (MSC) and maintained in the *in vitro* culture collection of the Institute of Biology Bucharest, Romanian Academy.

Experimental conditions. The initial explants, represented by 0.5g fragments of pale red callus from *V. vinifera* cv. Negru Vârtos long-term callus culture were spread on 0.5 cm surface of MSC medium variant (MS with NAA and kinetin) in each the Petri dish (6 cm diameter).

Different stress conditions used in the experiment are represented in Table 1. The stress conditions were induced by adding different chemicals to the MSC medium or by applying different physical factors at various exposure times.

Table 1. Stress conditions used for secondary metabolites induction in *V. vinifera* cv. Negru Vârtos long-term callus

	Growth conditions/Time	Variants
Control	MS/ 3 weeks	**A**
Chemical	MSC+CaCl2 · $2H_2O$ (440 mg/L) / 3 weeks	**B**
	MSC+NaCl (50 mM) / 3 weeks	**R1**
	MSC+NaCl (100 mM) / 3 weeks	**R2**
Control	MSC/ 3 weeks	**M**
Physical	MSC + 4°C / 24 h	**T1**
	MSC + 4°C / 48 h	**T2**
	MSC + UV exposure / 10 minutes	**U1**
	MSC + UV exposure / 15 minutes	**U2**

After exposure to different stress conditions, the callus was subcultivated on the MSC medium variant and maintained under *in vitro* conditions at 24 ± 2°C of temperature and 16/8 h photoperiod. After 4 weeks of subcultivation, the following morphometrical parameters were registered:

- callus survival percentage expressed after the formula: % survival = (number of developed calli / total number of inocula) × 100 %;
- callus fragment growth (cm) was quantified by measuring the diameter of the developed callus;
- callus growth index (CGI) was calculated as $CGI = (W_f - W_i)/W_i \times 100$ (Sahraroo et al., 2014) where W_i and W_f are the fresh weights registered at the beginning and the end of the growth period (4 weeks)
- fresh weight (FW, g) of the callus was recorded at 4 weeks, and dry weight (DW, g) was determined by incubation of a fresh callus sample in an oven at 55°C until the constant weight was reached;
- moisture content was calculated using the formula: Moisture content % = [(FW - DW) / FW] × 100.
- callus color intensity using a visual scale of yellow, red pale, and intense red.

Biochemical analysis

Extract Preparation. For extract preparation, 1 gram of *V. vinifera* callus (fresh weight) was crushed using a mortar and pestle, then 5 ml of methanol was added, and the samples were vigorously vortexed for 1 minute at room temperature. Homogenized samples were placed on an orbital shaker (Heidolph Instruments GmbH, Germany) and extracted for 2 days under continuous shaking, in the dark, at room temperature (RT). After this, samples were centrifuged (15 min, 12000 rpm, 4°C) using an Eppendorf centrifuge (Germany). The supernatant was transferred into a clean tube and stored at 4 °C.

Evaluation of Total Phenolic Content (PC). Total phenolic content was determined using the Folin-Ciocalteu method (Stankovic et al., 2011). Briefly, 2.5 ml Folin Ciocalteu solution and 2 ml Na_2CO_3 7.5% were added to 0.5 ml of diluted extract. The samples were incubated (30 min, RT) and the absorbance was measured at 765 nm and compared to the blank prepared with methanol. Results were expressed as mg of gallic acid equivalents (GAE)/g of DW, based on a calibration curve (10-100 µg/mL, $R^2 = 0.9968$).

Evaluation of Total Flavonoid Content (FC). Total flavonoid content was measured using a protocol adapted from Cai et al. (2012). In brief, 0.5 ml of dried extract was mixed with 2 ml distilled water and 0.15 ml 5% $NaNO_2$. The mixture was incubated (5 min, RT), then a 10% $AlCl_3$ solution was added. After 6 min at RT, 2 ml of 4% NaOH (m/v) and 1.2 ml distilled water were added to reach a final volume of 5 ml. The samples were stirred (15 min, RT) and the absorbance for each sample was read at 510 nm and compared to the blank. Results were expressed as mg of rutin equivalents (RE)/g of DW, based on a calibration curve (100-1000 µg/ml, $R^2 = 0.9996$).

Evaluation of Proanthocyanidin Content (PAC). The content of proanthocyanidins was determined according to Sun et al. (1998). Briefly, 400 µl of diluted extract was mixed with 3 ml of 4% vanillin solution in methanol (m/v) and 1.5 µl of hydrochloric acid. After 15 min incubation, 5 ml of glacial acetic acid was added, and the absorbance was read at 500 nm and compared to blank. Results were expressed as milligrams of catechin equivalents (CE)/g of DW, based on a calibration curve (20-200 µg/ml, $R^2 = 0.9924$).

Evaluation of Total Anthocyanins Content (TAC). Total anthocyanin content was determined using the standard method (Pasqualone et al., 2015). The dilution samples were prepared in methanol - 2N HCl solution (85:15 v/v). The absorbance of each sample was measured at 520 nm against a blank prepared with methanol - HCl. The absorbance of each sample was calculated according to the following formula:

Anthocyanin conc. (mg/g DW) = (A × MW × DF × 1000)/(ε × 1)

where: MW = molecular weight (449.2 g/mol C-3-G); DF = dilution factor; 1 = optical path (1 cm); ε = molar absorptivity (26,900 L × mol^{-1} × cm^{-1} for C-3-G).

Results were expressed as mg cyanidin-3-glucoside equivalents (C-3-GE)/g DW.

Evaluation of Antioxidant Activity Through DPPH Assays. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was applied according to Marxen et al. (2007). In brief, 2.25 ml methanol was mixed with 0.1 ml of diluted extract and 0.150 ml DPPH solution (1.27 mM). The reference and blank samples were prepared by mixing 2.35 ml methanol with 0.15 ml DPPH and 2.4 ml methanol with 0.1 ml diluted extract, respectively. Samples were incubated (30 minutes, RT) in the dark, and the absorbance was measured at 515 nm and compared to the reference sample. Results were expressed as mM of Trolox equivalents (TE)/g of DW, according to a standard curve (50-150 µg/ml, $R^2 = 0.9873$.

Statistical analysis. All the experiments consisted of 15 samples/treatments and the experiment was 3 times repeated. The results are reported as the means ± SD.

Before statistical analysis, the Shapiro-Wilk test was used to determine the normality of the dataset. The significant result ($p < 0.05$) of the test indicated the necessity to apply non-parametric techniques.

The Kruskal-Wallis test is a non-parametric method used to determine if there are statistically significant differences between two or more independent groups. If the test indicated significant differences between groups, pairwise comparisons were applied

using Dunn's post hoc test. To estimate the test distribution, the Monte Carlo method with 10,000 simulations was used. The technique is an alternative to the one-way ANOVA analysis applied when the normality of data conditions is not met (Ostertagova et al., 2014).

To analyze the relationship between bioactive compounds and antioxidant activity, the Partial Least Squares (PLS) regression method was used. This method was chosen due to its efficiency in managing data sets, allowing the identification of relationships between the independent variables (bioactive compounds) and the dependent variable (DPPH antioxidant activity). PLS models are particularly recommended for data sets with correlated variables, as they generate a set of components that best explain the dependent variable. The following coefficients were selected from the analysis*: Coefficients of determination* (R^2Y and R^2X) - R^2Y indicates the proportion of the variation in the dependent variable (Y) explained by the model. An R^2Y close to 1 suggests a good model fit. R^2X measures how well the PLS components explain the independent variables (X). A high R^2X suggests a good representation of the explanatory variables by the model components, *Predictive power* (cumulative Q^2), Q^2 (how) is a parameter that indicates the ability of the model to make predictions for new data. A Q^2(cum) close to 1 suggests high predictive power and *Variable Importance in Projection (*VIP) is an indicator of the contribution of each independent variable to the model. Variables with VIP > 1 are considered essential for prediction, and those with VIP < 0.8 can be eliminated to simplify the model (Tenenhaus et al., 2005).

Statistical analyses were performed with the XLSTAT software (2013).

RESULTS AND DISCUSSIONS

In the context of increasing demands for obtaining plant biomass with high secondary metabolite content, which can be exploited in various industries, the use of *in vitro* cultures for *V. vinifera* cv. Negru Vârtos was fully justified.

Previously, various studies were conducted regarding the isolation of *V. vinifera* callus lines with high proliferative capacities and secondary metabolites production (Brezeanu et al., 1993a; Brezeanu et al., 1999b; Lupşea & Brezeanu, 1999; Cogălniceanu at al., 2000a; Matienco et al., 2004; Mihai et al., 2009; Mihai et al., 2010; Mihai et al., 2013). Different factors like light intensity, KH_2PO_4 or NH_4NO_3 depletion (Karaaslan et al., 2013) and different elicitors such as salicylic acid, abscisic acid, jasmonic acid or mannitol (Mihai et al., 2010) were tested for increased the anthocyanin accumulation, the phenolic content and antioxidant activity in the grape callus lines.

In this study, two new chemicals ($CaCl_2 \cdot 2H_2O$ excess and NaCl) and two physical factors (low temperature and UV) to induced stress conditions were tested for elicitation of secondary metabolites as polyphenols, proanthocyanins, anthocyanidins, and flavonoids accumulation in long-term callus cultures of *V. vinifera* cv. Negru Vârtos.

The morphometrical parameters of the long-term callus cultures of *V. vinifera* cv. Negru Vârtos exposed to these stress conditions are illustrated in Table 2.

The various types of abiotic stress did not affect the callus survival, except for the UV treatments which determined a decrease in the survival rates to 84.13% (for U2 variant). The Kruskal-Wallis test (χ^2; = 29.7335; df = 7, p = 0.0002) indicated a significant difference between the analyzed groups in terms of survival rates. Dunn's post hoc pairwise comparison analysis reveals several significant differences. Thus, samples exposed to ultraviolet radiation for 10 and 15 minutes – the U variants) were clearly distinguished from the other groups, differing significantly (p = 0.0082 and p = 0.0004) from all other variants tested.

The callus growth index (CGI) and growth area were used to quantify the 3-D callus growth in surface and weight. The callus growth index was more affected by chemical treatments, unlike short treatments with physical stressors. All variants treated with $CaCl_2 \cdot 2H_2O$ in excess or NaCl registered a decrease in callus growth indexes than control, with the lowest value of 53.27% for the R1 variant treated with 50mM NaCl (Table 2). In the case of physical stressors the T1 and U2 variants showed increased values for callus growth index than control, indicating that these treatments induced callus growth.

Table 2. Morphometrical parameters of the long-term callus cultures of *V. vinifera* cv. Negru Vârtos exposed to different stress conditions

Stress variants	Survival percent (%)	Callus growth index (%)	Callus growth area (cm)	Moisture (%)	Callus color intensity
A	100	69.87	2.02 ± 2.76	98.53	Red
B	100	66.88	1.37 ± 0.51	98.48	Pale red
R1	100	53.27	2.56 ± 3.17	98.23	Intense red
R2	100	60.62	1.75 ± 0.41	98.24	Intense red
M	100	80.31	2.02 ± 2.76	98.36	red
T1	100	81.41	2.68 ± 1.61	98.54	Pale red
T2	100	75.12	3.3 ± 1.31	98.26	Pale red
U1	94	74.48	2.26 ± 1.17	98.48	Pale red
U2	84.13	82.01	2.29 ± 1.15	98.36	Pale red

The callus growth index was not associated with callus diameter growth (cm), which varied between 1.37 ± 0.51 cm for the B variant (excess of $CaCl_2 \cdot 2H_2O$) to 3.3 ± 1.31 cm for the T1 variant (4°C for 24 hours). These results suggested that the callus exposed to low temperature was more stressed, with callus cells being conditioned to spread on a medium surface to be in contact with the nutrients from the substrate.

Fresh and dry weight are two very important measurable variables that provide precise measurements of produced biomass. Based on the Kruskal-Wallis test and Dunn's post hoc pairwise comparisons, no statistically significant differences were found between the tested treatments analyzed in terms of fresh weight. Maintaining adequate moisture is crucial for the proper functioning of enzymes and the transport of nutrients within callus cells (Saurabh, 2015). In our case, the high moisture content was associated with the high survival rate of the callus, the stress conditions tested did not cause water accumulation in the cells. In their study, Mihai et al. (2013) underlined that high rates of biomass did not affect the phenolics and flavonoids production.

After 4 weeks, the calli maintained on the control variant (A) were almost red (100%), the calluses exposed to excess $CaCl_2 \cdot 2H_2O$ (B variant) were pale red, the calli subjected to excess salt (R variants) were intense red, while the calli subjected to low temperature (T variants) and UV (variants U) showed a pale red color. Under the red callus, in contact with the medium, islands of green callus were observed, especially in T variants (Figures 1 and 2).



Figure 1. *V. vinifera* cv. Negru Vârtos long-term callus initial explant

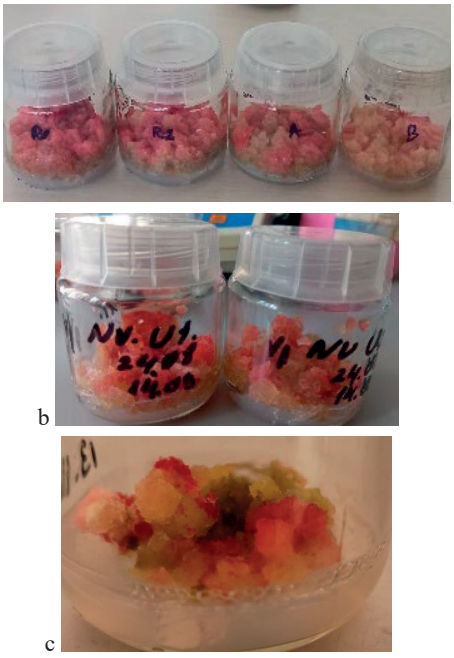


Figure 2. *V. vinifera* cv. Negru Vârtos long-term callus after stress experiment (a - R1, R2, A, B variants; b - UV variants; c - T1 variant)

Different abiotic stress was tested to induce secondary metabolite accumulation in long-term callus cultures of *V. vinifera* cv. Negru Vârtos.
Comparing different stress conditions, the Kruskal-Wallis test revealed significant differences between the variants analyzed for each of the studied compounds ($p < 0.0001$).
The highest accumulation of polyphenols was registered in unstressed variant M (control) and varied between stress conditions, from the highest content in the callus exposed to 4°C for 24 hours (T1 variant) (36.90 mg GAE/g DW) and the lowest content (11,757 mg GAE/g DW) in the callus exposed to an excess of $CaCl_2 \cdot 2H_2O$ (Figure 3a). For polyphenols ($\chi^2 = 31.49$), df = 7; $p < 0.0001$), the callus sample exposed to 4°C for 24 hours (T1 variant) was significantly different from the B variant ($CaCl_2 \cdot 2H_2O$ in excess) ($p < 0.0001$) but also beside the other variants exposure to the stress conditions. These results suggested that the T1 variant was the least affected option.
Concerning proanthocyanidins accumulation, callus exposure to 4°C for 24 hours (T1 variant) showed a slight increase in control (Figure 3b). Also, this variant had significant differences from B (excess $CaCl_2 \cdot 2H_2O$) ($p < 0.0001$) and R1 (50 mM NaCl) ($p = 0.0007$) variants.
In the case of anthocyanins, significant differences were recorded between variants ($\chi^2 = 28.25$; df = 7; $p < 0.0001$), the most notable being between the M variant (Control) and T1 variant ($p = 0.0006$), this treatment with low temperature stimulated synthesis of the anthocyanins (Figure 3c).
The flavonoid content varied between 16.39 (R1 variant) and 91.98 mg RE/g DW (T1 variant), the most significant differences were observed between T1 and B ($p = 0.00$), R1 ($p < 0.0001$), R2 ($p < 0.0001$) variants. Again, T1 was the variant treatment with the most differences from other variants and control, indicating a significant influence of low temperature on flavonoid content (Figure 3d). Our results are superior to Cetin's (2014) where a maximum total flavonol content (7.12 mg) was obtained in Öküzgözü callus exposed to UV for 5 min.

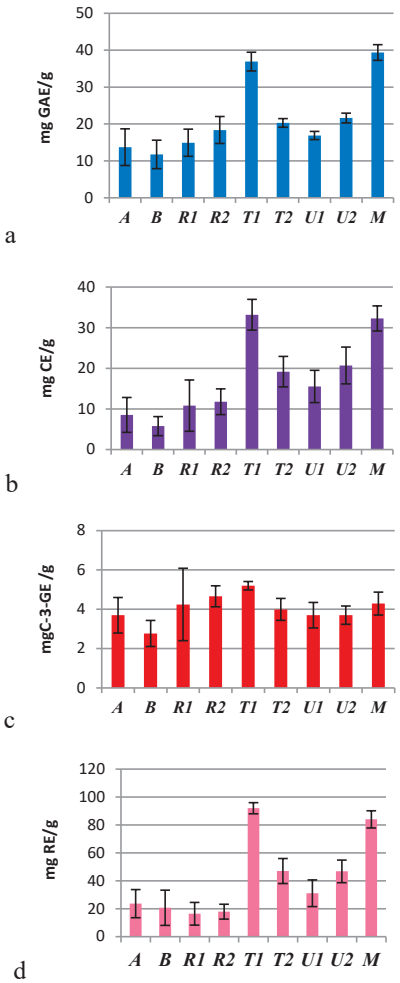


Figure 3. Secondary metabolites content in *V. vinifera* cv. Negru Vârtos long-term callus after exposure to different stress factors, polyphenols (a), proanthocyanidins (b), anthocyanidins (c), and flavonoids (d)

The antioxidant activity varied between 32.77-137.82 mM TE/g DW, but none of the stressed variants outperformed the control variants. The T1 variant (exposure for 24 hours at 4°C) showed significant differences from the B ($p < 0.0001$) and R1 ($p = 0.0001$) variants. Also, differences were observed between U2 and B ($p < 0.0001$), and B and R2 ($p = 0.001$) variants (Figure 4).

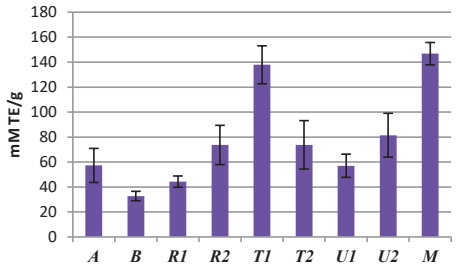


Figure 4. Antioxidant activity in *V. vinifera* cv. Negru Vârtos long-term callus after stress experiment

Compared with the control for both stress conditions (chemical - A and physical - M) no significant differences were observed, only a slight increase in the case of anthocyanidins, and flavonoids in the case of callus from the T1 variant.

The relationships between antioxidant activity and secondary metabolites tested analyzed through Partial Least Squares Regression (PLS Regression), highlighted their role in determining the antioxidant activity, with important variations between the types of samples, depending on the influence of each class of bioactive compounds. Analysis of PLS regression equations reveals how each bioactive compound influences DPPH antioxidant activity. In most samples, proanthocyanidins and polyphenols had either a positive or negative influence on DPPH, suggesting a variable impact on antioxidant activity. Flavonoids showed both positive and negative effects, but in general, their contribution was less compared to the other compounds. Anthocyanins had a different impact depending on the variants tested, in some cases having the strongest positive effect on DPPH activity and in others contributing negatively. Overall, polyphenols and proanthocyanidins are the most important factors in determining DPPH activity, while flavonoids and anthocyanins contribute to a variable extent (Table 3).

Table 3. PLS Regression Results: Q^2, R^2, and VIP Values for Evaluating Model Performance and Variable Importance

Variants	Q^2	R^2Y	R^2X_c	VIP			
				Proanthocyanidins	Polyphenols	Flavonoids	Anthocyanidins
A	0.78	0.82	0.98	0.86	1.07	1.04	1.02
B	0.58	0.72	0.71	1.35	1.25	0.35	0.71
R1	0.54	0.61	0.99	1.01	1.02	0.95	1.02
R2	0.67	0.74	0.97	1.12	0.87	1.08	0.88
T1	0.90	0.93	0.74	1.17	1.10	0.42	1.11
T2	0.98	0.99	0.73	1.15	0.84	1.17	0.77
U1	0.99	0.99	0.92	1.04	1.01	1.04	0.90
U2	0.93	0.94	0.90	0.77	1.02	1.09	1.09

Legend: Q^2 - Predictive power; R^2Y and R^2X - Coefficients of determination; VIP - Variable Importance in Projection

To identify the composition of the callus growth medium variant, it must be taken into account that in the process of growth and development of the callus, there are 2 types of cells (those at the base, colorless, which help the growth, and those at the surface, colored, characterized by the accumulation of secondary metabolites (Deroles, 2009). Balancing the nutrient composition to obtain both growth and secondary metabolites is the desired task. Knowing that calcium chloride ($CaCl_2 \cdot 2H_2O$) is often used in viticulture to improve fruit quality, we tested an excess concentration. Calcium, one of the essential nutrients, plays crucial roles in cell wall structure, enzyme activation, and signal transduction (White et al., 2003). An excessive application of $CaCl_2 \cdot 2H_2O$ can have a significant impact on the secondary metabolite profile of *V. vinifera* (Martins et al., 2021), fact was also proven in our case (2 times $CaCl_2 \cdot 2H_2O$ concentration - B variant, compared to the control variant), where all the secondary metabolites analyzed in the callus were in reduced quantity compared to the control, but also to the other stress variants (Figure 3).

Salt stress can induce the accumulation of phenolic compounds, such as flavonoids (anthocyanins, flavonols), with antioxidant effects protecting cells from damage caused by salt stress (Almagro et al., 2022). Salt stress can also alter the relative proportions of different secondary metabolites, affecting the ratio of anthocyanins to flavonols and influencing color. In our case, the callus subjected to 50-100 mM NaCl (R variants) was characterized by a color intensification of the cells (higher concentration, higher intensification), which proves a higher anthocyanin load compared to the control (Figure 3).

A 24-hour exposure of long-term callus cultures of *V. vinifera* cv. Negru Vârtos at 4°C (T1 variant) assures the highest secondary metabolite accumulation among all the tested variants. Kumar et al., 2023 showed that grapevines can trigger the production of certain secondary metabolites as a defense mechanism in response to cold stress. Prolonged cold exposure may have detrimental effects (Ramakrishna & Ravishankar, 2011), in our case, the 48-hour exposure (T2 variant) reduces almost half the secondary metabolites accumulation.

UV radiation has been shown to have the most significant impact on secondary metabolite production in grapevines, triggering various physiological responses, leading to the accumulation of beneficial compounds (Del-Castillo-Alonso et al., 2021); enhancing the phenolic compounds (e.g., anthocyanins, flavonols) biosynthesis, compounds with contribution to the color, astringency, and antioxidant properties of grapes and wine (Cozzolino, 2025).

The presentations of secondary metabolites analyzed in both fresh and dry-weight will be helpful, in future research, to normalize the data, determine water-soluble vs. water-insoluble compounds, understand the metabolite accumulation, track the metabolite accumulation, optimize extraction methods, etc. (Oancea et al., 2025)**.**

CONCLUSIONS

The results of these studies could have important implications for the production of valuable secondary metabolites from *V. vinifera*. By understanding the factors that influence the accumulation of secondary metabolites in callus cultures, it may be possible to develop more efficient methods for producing these compounds for use in the pharmaceutical, food, and cosmetic industries.

Between treatments, the cold storage at 4°C for 24 hours (T1 variant) ensures not only the biomass growth but also triggers the accumulation of secondary metabolites of interest in a higher quantity, as anthocyanidins and flavonoids.

In perspective, the combined testing of abiotic factors and different protocols for stress induction will be considered.

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COMPARISON OF THE PHENOLIC COMPOSITION OF MAMAIA, BĂBEASCĂ NEAGRĂ AND PINOT NOIR BLACK GRAPES IN THE MURFATLAR WINE CENTER

Iulia-Cristina CIOBANU[1], Traian Ştefan COSMA[1], Victoria ARTEM[1], Arina Oana ANTOCE[2]

[1]Research Station for Viticulture and Oenology Murfatlar, 2 Calea Bucuresti, Murfatlar, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: arina.antoce@horticultura-bucuresti.ro

Abstract

Grape polyphenols are essential components that determine the quality and benefits of derived products, contribute to organoleptic characteristics (color, aroma, taste) and have remarkable antioxidant properties. The research aimed to compare black grape varieties from Murfatlar wine center to assess if their winemaking pomace is a good polyphenols source for food fortification. The varieties Mamaia, Băbeasca Neagră and Pinot Noir were especially selected, as they are not intensely coloured varieties and are usually not valorized for polyphenol extraction. During 2024 the grape ripening process was monitored and the must and the fresh, unfermented, pomace were analysed using the ITV method. The following parameters were determined: total polyphenol content, extractable anthocyanin concentration, total anthocyanin potential, total polyphenol index and antioxidant activity. The results indicated that Pinot Noir exhibited the highest values, with total polyphenols of 1022.3 mg GAE/L, 385.4 mg/L anthocyanins, and 36.2% antioxidant activity, followed by Băbeasca Neagră and Mamaia. These findings highlight the remarkable potential of Pinot Noir for pomace utilization, while also demonstrating the usability of the other varieties in enhancing the valorization of winemaking by-products.

Key words*: grape pomace, antioxidant activity, fortified foods, anthocyanins.*

INTRODUCTION

Grape polyphenols are essential bioactive compounds that play a pivotal role in defining both the quality and the health-promoting properties of grape-derived products (Zhou et al., 2022). These compounds significantly influence the organoleptic characteristics of grapes and their derived products, such as colour, aroma, and taste - attributes that are critical for consumer acceptance and market value (Lesschaeve et al., 2005; Gutiérrez-Escobar et al., 2021). In addition to their sensory contributions, grape polyphenols exhibit remarkable antioxidant properties, which are essential in mitigating oxidative stress and preventing the onset of various chronic diseases (Rudrapal et al., 2022). Their multifunctional role not only enhances the sensory appeal of grape-based beverages and foods (Kandylis et al., 2021) but also underscores their relevance in the development of nutraceuticals and functional food products (Caleja et al., 2017). As a result, grape polyphenols and their applications have become a prominent focus of research, aiming to maximize their potential through innovative processing and preservation techniques.

In recent years, the valorisation of winemaking by-products, particularly grape pomace, has received increasing attention due to its richness in bioactive compounds (Karastergiou et al., 2024; Wang et al., 2024), including polyphenols, anthocyanins, and other antioxidants. Grape pomace - composed of skins, seeds, and stems remaining after juice extraction - represents a significant, yet underutilized, resource in the food industry. Current research emphasizes that polyphenols extracted from grape pomace can be effectively used in food fortification (Sridhar et al., 2021), contributing to the creation of functional foods with enhanced health benefits (Eliopoulos et al., 2022). Furthermore, the extraction and

characterization of these compounds from various grape varieties provide valuable insights into their potential applications across multiple industries, including food, pharmaceutical, and cosmetic sectors.

In Romania, the Murfatlar wine centre is renowned for its viticultural tradition and diverse grape assortment. However, despite the significant production of red grape varieties, many of them remain insufficiently explored regarding their polyphenolic composition and potential uses beyond traditional winemaking. This study focuses on three black grape varieties cultivated in the Murfatlar wine centre: Mamaia, Băbească Neagră and Pinot Noir. These varieties were chosen because of their relatively low coloration compared to other intensely pigmented red cultivars, which often leads to their limited valorisation for polyphenol extraction.

The primary objective of this research is to compare the polyphenolic profiles of these selected grape varieties to assess their suitability for further applications, particularly in the context of winemaking by-product valorisation. The study evaluates key parameters, including total polyphenol content, extractable anthocyanin concentration, total anthocyanin potential, total polyphenol index, and antioxidant activity. Through this analysis, the research aims to provide a comprehensive evaluation of the potential of these grape varieties for food fortification and other functional uses.

Given the growing demand for sustainable and health-promoting ingredients in the food industry, this study intends to contribute valuable knowledge to the field of grape by-product utilization. The findings are expected to support the development of innovative strategies to enhance both the economic and nutritional value of winemaking residues, thereby encouraging a more sustainable and circular approach to viticulture and food production.

MATERIALS AND METHODS

For this study three red wine grape varieties were considered, which have not been the focus of previous research: the Mamaia variety, developed at the Murfatlar Research and Development Station, a Romanian-specific variety, Băbească Neagră, and an international variety, Pinot Noir.

For the first part of this study, focusing on grape sample analysis, grapes were collected weekly between August 22 and September 16, 2024. In order to determine the polyphenolic potential, the ITV method was applied. For the extraction 200 grape berries of each variety were crushed, and samples of 50 grams of the resulting mix were taken for analysis. The samples were treated with 85 ml of 0.1% HCl and 15 ml of 96% ethanol. After homogenization, the extraction was carried out at room temperature for one hour, with the sample being stirred every 15 minutes. Subsequently, the mixture was filtered, and the specific analyses were performed on the liquid fraction.

The determinations were performed spectrophotometrically at wavelengths of 280 and 520 nm, using a 1 cm optical path cuvette, against distilled water, with the Metertech SP-8001 spectrophotometer.

For the second part of the study, focusing on grape pomace analysis, the folowing procedure was used:

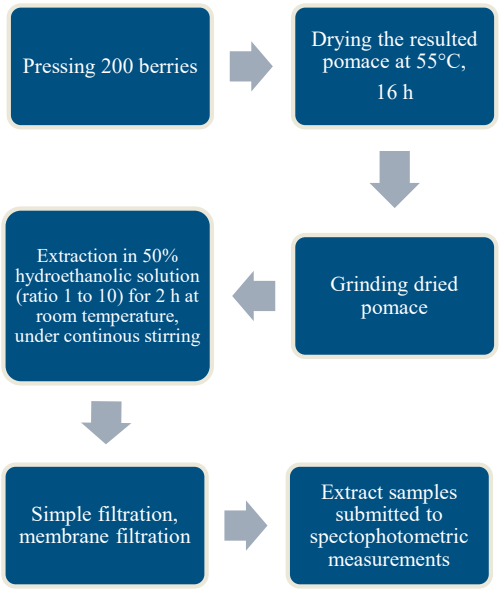


Figure 1. Technique for obtaining the pomace extracts analysed in the study

The obtained extracts subsequently underwent a series of spectrophotometric determinations, such as the total polyphenol index, anthocyanins, total polyphenols (Folin-Ciocalteu method, readings performed at 760 nm wavelenght), colour intensity, total anthocyanic potential and antioxidant activity (DPPH inhibition method, readings performed at 515 nm wavelenght). For the determination of total polyphenols and antioxidant activity, 1:10 dilutions of the extract were required. The results presented represent the average of three determinations. Standard errors were calculated and the means were compared for the 3 varieties by ANOVA and post-doc Tukey test $p<0.05$. For the statistical analysis the software Origin ver 9 was used.

RESULTS AND DISCUSSIONS

Determination of Phenolic Maturity Dynamics for Grapes

Phenolic maturity refers to the developmental stage of phenolic compounds present in grapes, primarily anthocyanins (pigments), proanthocyanidins (condensed tannins) and other relevant polyphenols, which directly influence grape quality. Figure 2 illustrates the dynamics of total polyphenol index evolution during the month before harvesting for the three grape varieties: Mamaia, Pinot Noir, and Băbească Neagră.

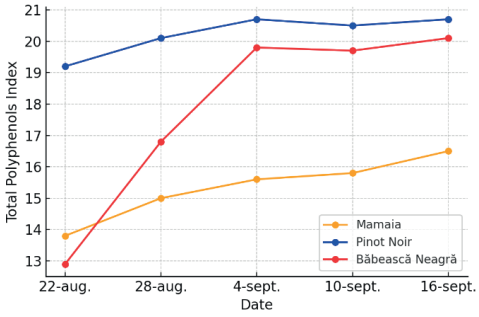


Figure 2. Total Polyphenols Index Dynamics for Grape Samples

The evolution of the Total Polyphenols Index for the three grape varieties shows distinct trends over the analysed period. Pinot Noir maintains the highest and most stable polyphenol content, ranging between 19.2 and 20.7, indicating an early and consistent accumulation pattern. Băbească Neagră shows a significant and steep increase, from 12.9 to 19.8 during the first two weeks of evaluation, followed by a steady phase in the last two weeks, in which there is a small progress from 19.8 to 20.1, suggesting a strong accumulation of polyphenols during ripening, reaching similar levels to Pinot Noir by mid-September. Mamaia has the lowest total polyphenols content but shows a gradual increase from 13.8 to 16.5, indicating a moderate accumulation capacity compared to the other two varieties.

The dynamics of anthocyanin accumulation (Figure 3) shows clear differences among the three grape varieties. Pinot Noir maintains the highest and most stable anthocyanin levels (92.4-95.1 mg/L), indicating early and steady accumulation. Băbească Neagră shows a significant and continuous increase during the entire month previous to harvesting, from 51 mg/L to 106.8 mg/L, suggesting a late and dynamic accumulation pattern. Mamaia has the slowest and most stable anthocyanin content evolution, with only a slight increase (53.7 to 59.3 mg/L), followed by a minor decrease.

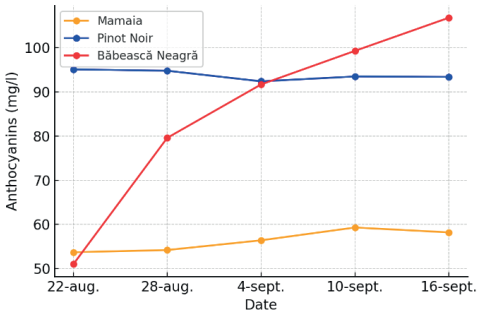


Figure 3. Dynamics of Anthocyanins Accumulations for Grape Samples

Of the three varieties, Pinot Noir is rich in anthocyanins and has a stable anthocyanin content in the month previous to harvest, consistent with the fact that it is a grape with early maturation. Băbească Neagră accumulates intensively over time in the month before harvesting, reaching higher concentrations of anthocyanins as compared to Pinot Noir. Of all studied varieties, Mamaia shows a lower anthocyanin synthesis potential,

progressing slowly during the month before harvesting and reaching a maximum of 60 mg/L.

The analysis of total polyphenols and anthocyanins accumulation dynamics shows distinct phenolic maturity patterns among the studied grape varieties. Pinot Noir is characterized by an early and consistent accumulation of both total polyphenols and the specific class of anthocyanins, maintaining high and stable levels throughout the ripening period. In contrast, Băbească Neagră shows a significant and continuous increase in both total polyphenols and anthocyanins, indicating a late but intense accumulation process that leads to high final concentrations. Meanwhile, Mamaia presents the lowest accumulation capacity for both measured parameters, with only moderate increases in polyphenols and minimal variation in anthocyanin content, suggesting a limited phenolic maturation potential compared to the other varieties.

Assessment of the polyphenolic profile of wine industry by-products (Grape Pomace extracts)

The extracts obtained from the grape pomace, resulted after winemaking following the methodology detailed in the 'Materials and Methods' section, were subjected to spectrophotometric analysis to evaluate their general polyphenolic composition and associated properties. For the assessment of total polyphenols and antioxidant activity, a 1:10 dilution of the extract was used to ensure the accuracy and reliability of the measurements.

The polyphenolic profile and antioxidant activity of the analysed grape pomace samples (Table 1) reveal significant differences in the content and distribution of bioactive compounds, highlighting their potential for revalorization as sources of natural antioxidants and phenolic extracts.

Among the studied samples, Pinot Noir pomace exhibited the highest concentrations for most analysed parameters, indicating its superior potential as a raw material for total phenols or for anthocyanin recovery. The Total Polyphenols Index (338.1) reflects a high content of extractable polyphenolic compounds, in direct relation with the total polyphenol quantity (1022.3 mg GAE/L), while the anthocyanin content (385.4 mg/L) also confirms the presence of significant amounts of bioactive molecules. Furthermore, the total anthocyanic potential (1156.2 mg/kg) demonstrates a considerable resource of anthocyanins that could be efficiently extracted and utilized in the production of functional ingredients. The antioxidant activity of 36.2% reinforces the potential of Pinot Noir pomace as a rich source of compounds with good radical scavenging properties, suitable for applications in the food, pharmaceutical, and cosmetic industries.

Băbească Neagră pomace presented intermediate values, suggesting a balanced phenolic composition with potential for exploitation. Although its Total Polyphenols Index (290.4) and total polyphenols (741.5 mg GAE/L) are somewhat lower than those of Pinot Noir, the anthocyanin content (237.0 mg/L) and total anthocyanic potential (711.0 mg/kg) indicate the presence of a substantial amount of pigments and antioxidant compounds that could be valorised. Additionally, the antioxidant activity of 32.8% confirms the presence of effective antioxidant molecules, supporting the use of Băbească Neagră pomace in the development of natural extracts for functional products.

Table 1. Polyphenolic Profile and Antioxidant Activity of the Pomace for the Studied Grape Varieties

Variety	Total Polyphenols Index (DO 280 nm)	Anthocyanins (mg/L)	Total Polyphenols (mg GAE/L)	Colour Intensity (IC 420+520+620)	Total Anthocyanic Potential (mg/kg)	Antioxidant Activity (%)
Mamaia	312.0 ± 2.9^{a}	137.9 ± 3.2^{a}	875.0 ± 5.5^{a}	4.62 ± 0.01^{a}	413.7 ± 9.6^{a}	22.2 ± 2.5^{a}
Pinot Noir	338.1 ± 3.4^{b}	385.6 ± 4.2^{b}	1022.3 ± 8.3^{b}	6.64 ± 0.02^{b}	1156.2 ± 11.7^{b}	36.2 ± 2.7^{b}
Băbească Neagră	290.4 ± 2.2^{c}	237.0 ± 3.7^{c}	741.5 ± 5.2^{c}	6.23 ± 0.01^{c}	711.0 ± 11.1^{c}	32.8 ± 2.8^{b}

Mean values of three independent determinations ± standard deviations. The comparison of means was done by ANOVA and post hoc Tukey test. Different letters represent significant differences at $p<0.05$.

Although Mamaia pomace exhibited lower contents of polyphenols, anthocyanins, and antioxidant activity compared to the other varieties, it nevertheless represents a valuable resource for revalorization. With a Total Polyphenols Index of 312.0 and a total polyphenols content of 875.0 mg GAE/L, Mamaia pomace still contains significant amounts of phenolic compounds. Although the anthocyanin concentration of 137.9 mg/L and total anthocyanic potential of 413.7 mg/kg of Mamaia variety are lower than those observed in Pinot Noir and Băbească Neagră, these levels may still support the extraction of natural pigments and bioactive compounds. Furthermore, while the Mamaia's antioxidant activity of 22.2% was the lowest among the studied samples, it still highlights the presence of compounds with functional properties, thus making Mamaia pomace a potential raw material for applications where moderate antioxidant activity is desirable, contributing to the sustainable valorisation of winery by-products.

CONCLUSIONS

The present study provides a comparative assessment of the polyphenolic composition and antioxidant activity of grape pomace derived from three black grape varieties cultivated in the Murfatlar wine centre: Mamaia, Băbească Neagră, and Pinot Noir. The results clearly demonstrate the richness and diversity of bioactive compounds present in these underutilized by-products, supporting their potential for revalorization in functional applications.

Among the analysed varieties, Pinot Noir pomace exhibited the highest levels of total polyphenols, anthocyanins, total anthocyanic potential and antioxidant activity, highlighting it as a promising raw material for the extraction of natural antioxidants and pigments. These findings underline Pinot Noir's exceptional potential for applications in the development of fortified foods, nutraceuticals, and cosmetic formulations aimed at enhancing health benefits and product functionality.

Băbească Neagră pomace presented intermediate values for all analysed parameters, demonstrating a well-balanced polyphenolic profile with considerable anthocyanin and antioxidant content. This variety can be considered a valuable source of natural compounds for industries seeking bioactive-rich ingredients, particularly for functional food fortification and antioxidant supplementation.

Although Mamaia pomace showed the lowest polyphenolic and antioxidant levels among the studied varieties, it nonetheless contains significant amounts of polyphenolic bioactive compounds, including anthocyanins. These results suggest that Mamaia pomace, despite its lower phenolic content, remains an important resource for revalorization. Its exploitation contributes to a sustainable approach to winery by-products, preventing waste and creating added value.

Overall, this research emphasizes the importance of varietal selection when considering grape pomace as a raw material for polyphenol recovery. The study demonstrates that even less intensely coloured varieties, traditionally not valorised for their phenolic content, can serve as important sources of bioactive compounds, supporting circular economy principles in viticulture. Future research should focus on optimizing extraction techniques and exploring specific applications of these compounds, contributing to a more sustainable and innovative use of grape pomace.

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ASSESSMENT OF GENETIC DIVERSITY OF GRAPEVINE GENOTYPES USING SIMPLE SEQUENCE REPEATS (SSR) MARKERS AND SSR-BASED DNA BARCODE DESIGN

Monica HÂRŢA[1], Rodica POP[1], Liliana ROTARU[2], Lucia Cintia COLIBABA[2], Alin DOBREI[3], Steliana Paula BARBU[4], Doina CLAPA[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, Cluj-Napoca, Romania
[2]"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iași, Romania
[3]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului, Timişoara, Romania
[4]Valea Călugărească Research, Development Institute for Viticulture and Vinification, 2 Valea Mantei Street, Valea Călugărească, Romania

Corresponding author email: doina_clapa@usamvcluj.ro

Abstract

Romania has a valuable wine heritage, and the genetic evaluation of grapevine genotypes from Vitis germplasm collections is a national priority. Thus, this study aimed to characterize 22 Romanian grapevine genotypes and five international reference varieties using nine SSR (Simple Sequence Repeats) loci. VVMD32 was the most informative (He=0.837; PI= 0.040) SSR marker with 11 alleles generated. Conversely, the VvZag62 locus generated the lowest number of alleles (6) with values recorded for He=0.744 and PI= 0.109. The genetic SSR profiles were used to construct a barcode design to compare the number and size of alleles among the screened grapevine samples. The results of this study show that SSR genotyping supplemented with a useful data grouping mode is effective for genetic diversity analysis of Vitis germplasm resources.

Key words*: Vitis vinifera L., OIV microsatellites, DNA polymorphism, genetic resources.*

INTRODUCTION

Romania is a Southeastern European country with a long grapevine cultivation and winemaking tradition (Bodea et al., 2009). Over the centuries, the winemaking areas have been developed into favourable geographical areas for viticulture in Romania, depending on the relief, climatic conditions, and soil types (Soare et al., 2010; Bănuță et al., 2024).

Grapevine cultivation has existed since ancient time, and Romanian grape germplasm collections showcase various gene pools. These include autochthonous varieties, old local biotypes, and newly developed cultivars that have arisen from utilizing the genetic resources of both native and international varieties, interspecific hybrids, and wild grapevine populations of *Vitis vinifera* ssp. *sylvestris* (Popescu et al., 2018). Additionally, germplasm mixing occurred due to the import of different genotypes following the phylloxera epidemic (Pușcă, 2010). Ampelographic characterization and SSR molecular marker analysis of grapevine genotypes have been performed across various national germplasm collections in several European countries (Agar et al., 2011; Buhner-Zaharieva et al., 2010; Dzhambazova et al., 2009; Grando & Frisinghelli, 1998; Lefort & Roubelakis-Angelakis, 2000; Lopes et al., 1999; Martin et al., 2003; Pellerone et al., 2001; Štajner et al., 2011). These studies aimed to compile the results in the national *Vitis* databases or to coordinate efforts among them through the European *Vitis* database (Maul et al., 2012).The molecular evaluation of grapevine genotypes maintained in Romanian germplasm collections using SSR markers is still a work in progress. Some previously published studies (Gheorghe et al., 2010; Motoc et al., 2010) suggest that SSR markers are efficient and reliable tools for accurately characterising Romanian grapevine varieties. They complete the classical methods of identification based on

ampelographic description (Popescu & Crespan, 2018).
Identifying SSR genetic profiles for old local varieties and newly developed cultivars can serve as a passport certifying their authenticity. This approach also represents a critical strategy for the future preservation of Romanian grapevine accessions with scientific and economic significance (Ghețea et al., 2012; Ilina-Dumitru et al., 2024).
The current study utilized nine nuclear SSR loci to characterize 22 Romanian grapevine varieties, with five international cultivars included as comparative genetic profiles. The microsatellite analysis was employed to create a barcode system, a visual representation of the data that simplifies the detection of genotypic differences.

MATERIALS AND METHODS

Total genomic DNA isolation and PCR amplifications were carried out according to the protocol presented by Hârța & Pamfil (2013).
Table 1 presents the *Vitis vinifera* genotypes used for SSR genetic analysis.

Table1. Plant material used for SSR analysis

Genotype name	Berry colour	Geographic origin	Distribution code*
Alb aromat	white	Timiș county	330
Alb crocant	white	Timiș county	330
Alb lax	white	Timiș county	330
Arămiu	white	Timiș county	330
Auriu	white	Timiș county	330
Coada oii	white	Timiș county	330
Conic auriu	white	Timiș county	330
Gras aripat	white	Timiș county	330
Ochiul boului	white	Timiș county	330
Pătrunjarca	white	Timiș county	330
Răschirata	white	Timiș county	330
Cioinic	white	Iași county	320
Coarna albă	white	Iași county	320
Creața de Banat	white	Iași county	320
Cruciuliță	white	Iași county	320
Feteasca albă	white	Iași county	320
Feteasca regală	white	Iași county	320
Frâncușă	white	Iași county	320
Galbenă de Odobești	white	Iași county	320
Grasă de Cotnari	white	Iași county	320
Mustoasa de Măderat	white	Iași county	320
Zghihară de Huși	white	Iași county	320
Cabernet Sauvignon-RG**	white	Iași county	310
Muscat Ottonel-RG**	white	Iași county	310
Riesling italian-RG**	white	Iași county	310
Chardonay blanc-RG**	white	Iași county	310
Gewuerztraminer-RG**	rose	Iași county	310

In Table 1, the distribution codes were assigned according to Popescu & Crespan (2017): *310 = local cultivar, spread all over, international cultivar; 320 = major local cultivar, of local importance, but widely grown and 330 = minor local cultivar, of local importance, fairly grown; **RG denotes the reference genotypes.
Nine SSR loci were employed for SSR analysis: VVS2 (Thomas & Scott, 1993); MD5, MD7 (Bowers et al., 1996); MD25, MD27, MD28, MD32 (Bowers et al., 1999); VrZAG 62, VrZAG 79 (Sefc et al., 1999). These SSR markers are recognized as OIV (International Organization of Vine and Wine) descriptors (https://www.oiv.int/public/medias/6886/oiv-viti-609-2019-en.pdf).The features of the SSR primers used for analysing *Vitis vinifera* genotypes are displayed in Table 2.

Table2. Primer name and sequences used for SSR analysis

SSR primer name	Primer sequences (5`-3`)	OIV code
VVS2 F	CAGCCCGTAAATGTATCCATC	801
VVS2 R	AAATTCAAAATTCTAATTCAACTGG	801
VVMD5 F	CTAGAGCTACGCCAATCCAA	802
VVMD5 R	TATACCAAAAATCATATTCCTAAA	802
VVMD7 F	AGAGTTGCGGAGAACAGGAT	803
VVMD7 R	CGAACCTTCACACGCTTGAT	803
VVMD 27 F	GTACCAGATCTGAATACATCCGTAAGT	804
VVMD 27 R	ACGGGTATAGAGCAAACGGTGT	804
VrZAG 62 F	GGTGAAATGGGCACCGAACACACGC	805
VrZAG 62 R	CCATGTCTCTCCTCAGCTTCTCAGC	805
VrZAG 79 F	AGATTGTGGAGGAGGGAACAAACCG	806
VrZAG 79 R	TGCCCCCATTTTCAAACTCCCTTCC	806
VVMD 32 F	TATGATTTTTTAGGGGGGTGAGG	807
VVMD 32 R	GGAAAGATGGGATGACTCGC	807
VVMD 25 F	TTCCGTTAAAGCAAAAGAAAAAGG	808
VVMD 25 R	TTGGATTTGAAATTTATTGAGGGG	808
VVMD 28 F	AACAATTCAATGAAAAGAGAGAGAGAGA	809
VVMD 28 R	TCATCAATTTCGTATCTCTATTTGCTG	809

The sizes of alleles/ locus were evaluated using the CEQ fragment analysis software. Genetic parameters, including allele frequencies, expected and observed heterozygosity, estimated null allele frequencies, and the probability of identity, were calculated using Identity 1.0 software (Wagner & Sefc, 1999). DNA barcodes were organized in Microsoft Excel according to the protocol described by Galbacs et al. (2009).

RESULTS AND DISCUSSIONS

Nine SSR primer pairs amplified 77 SSR alleles, with an average of 8.55 alleles per locus, comparable to results obtained in previous reports (Coste et al., 2010; Carimi et al., 2010). The highest number of alleles was for VVMD32 locus (11), while the lowest for VrZAG62 locus (6). To evaluate the efficiency of microsatellite markers, we examined observed (Ho) and expected heterozygosity (He) to assess the

genetic variability among the analysed grapevines. Ho varied from 0.744 for VrZag62 to 0.868 for VVMD28. VVMD28 was the most informative SSR marker (He=0.868; PI= 0.031). Conversely, VvZag62 locus generated the lowest number of alleles (6) with recorded values for He=0.744 and PI= 0.109. The mean of observed heterozygosity (Ho) was slightly higher (0.826) than the expected value (0.802), with significant differences observed locus by locus (Table 3).

Table 3. Genetic parameters highlighting the genetic variability among the 27 grapevine genotypes

Locus	Allele size range (bp)	No. of alleles	He[1]	Ho[2]	Estimated frequency of null alleles	PI[3]
VVS2	133-153	8	0.778	0.629	+ 0.083	0.078
VVMD5	228-248	9	0.831	0.814	+ 0.008	0.048
VVMD7	235-257	8	0.773	0.740	+ 0.018	0.083
VVMD25	239-269	8	0.770	0.888	- 0.066	0.087
VVMD27	176-195	8	0.796	0.851	- 0.030	0.071
VVMD28	218-268	10	0.868	0.962	- 0.050	0.031
VVMD32	240-273	11	0.836	0.814	+ 0.011	0.040
VrZAG62	186-204	6	0.744	0.851	- 0.061	0.109
VrZAG79	237-259	9	0.831	0.888	- 0.031	0.044
Mean		8.55	0.802	0.826		

[1]Heterozygosity expected (He);
[2]Heterozygosity observed (Ho);
[3]Probability of identity (PI).

The mean observed heterozygosity (82.6%) was higher than that reported for other grapevine germplasm collections, such as Greece, with 73.7% (Bibi et al., 2020), and Turkey with 73.1% (Yüksel et al., 2023). It was also comparable to the values obtained by Lacombe et al. (2013) and Popescu & Crespan (2018). The alleles of the markers used in this study were evenly distributed among analysed genotypes, resulting in a low probability of identical genotypes (0.031-0.087). This was the case except for the VrZAG79 marker, which had a PI value of 0.109 as shown in Table 3. DNA microsatellite analysis was used to create a barcode design. This study's findings demonstrated that this data grouping system was effective in characterizing Romanian grapevine genotypes. The SSR barcode system visualizes data and facilitates the detection of genotypic differences. If an overlap occurs in the allele size representation (where two or more markers have the same allele sizes), the bar can be marked with an index indicating those differences (Galbacs et al., 2015; Chinnappareddy et al., 2012). In this study, the SSR results were converted into DNA barcodes by separating the allele sizes from each SSR locus and then sorting the allele size data from lowest to highest.

As shown in Figure 1, the allele size bars were created on a linear scale for all the analysed cultivars. This method of data grouping can be beneficial for further studies regarding the characterization of Romanian grapevine cultivars at the molecular level. Incorporating these DNA barcodes into a future Romanian-designed *Vitis* database could enhance the quality of genetic resource management in grapevines.

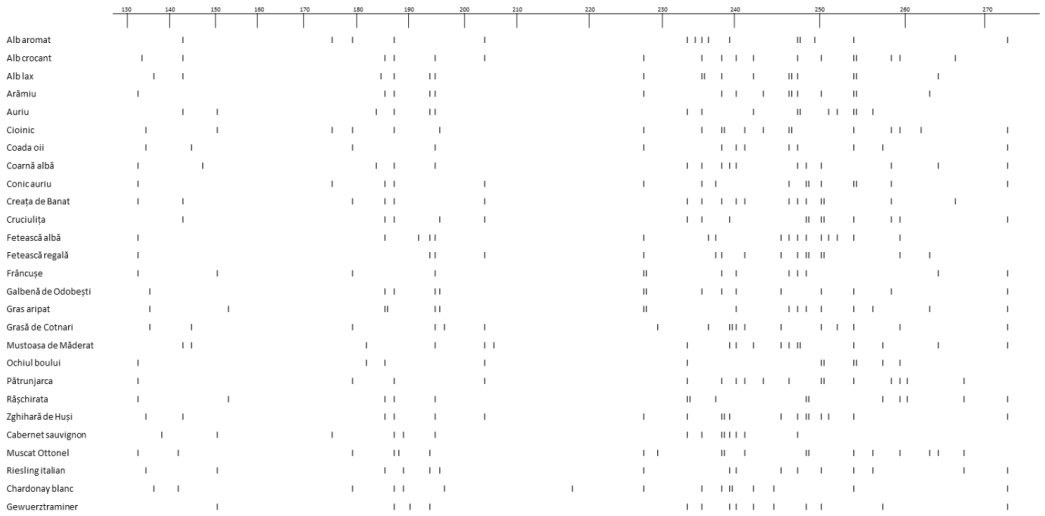


Figure1. SSR-based DNA barcodes of the studied grapevine genotypes

CONCLUSIONS

Although Romania has a valuable viticultural heritage, efforts to characterize grape varieties at the DNA level are still less emphasized compared to research results in other countries regarding molecular ampelography. In this study, the SSR genetic profiles of eleven *Vitis vinifera* genotypes (local biotypes) from Banat were identified for the first time, highlighting the genetic relationships with reference varieties and other Romanian varieties.

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TECHNOLOGICAL SOLUTIONS TO REDUCE THE DISTURBING EFFECT OF CLIMATE CHANGE IN THE DEALU MARE VINEYARD

Marian ION, Liliana PÎRCĂLABU, Cristian BURLACU

Research and Development Institute for Viticulture and EnologyValea Călugărească,
2 Valea Mantei Street, Valea Călugărească, Prahova, Romania

Corresponding author email: alexandrinalster@gmail.com

Abstract

The researches were carried out in the Valea Calugareasca viticultural center in a vineyard with the Cabernet Sauvignon variety. The experimental device included as an experimental factor the soil maintenance system (black field, permanent grass with herbs from spontaneous flora, lawn, phacelia, white clover and vetch). The viticultural climate, characterized by a high heliothermic regime, against the background of reduced water resources and the technological variants experienced had a differentiated impact on the quantity and quality of grape production. The soil maintenance variants with Phacelia and vetch ensured significant differences in production, +1.052 kg/vine and +0.924 kg/vine, respectively. The deviation from the optimal content of the sugar of 12.0-12.8% was registered in the case of the soil maintenance variant with Phacelia, vetch and white clover. The leaf area required to produce one gram of grape in the conditions of maximum sugar accumulations was between 11.05 cm^2/g grape (the soil maintenance system variant by phacelia) and 9.92 cm^2/g grape (the soil maintenance system variant as black field).

Key words*: climatic change; grapevine; phacelia; white clover; grape production.*

INTRODUCTION

In order to mitigate the disruptive effect of climate change in recent years, a series of agro-technical solutions have been experimented with, aimed at conserving water in the soil and reducing water loss through transpiration. These solutions focused on optimizing soil maintenance systems.

As a prolonged monoculture, the viticultural ecosystem generates a wide range of inconveniences, such as accentuated soil erosion on sloping terrain,with the loss of the fertile topsoil layer; reduction of soil organic matter content; degradation of the soil's physical, chemical, and biological properties, properties that form the basis of its fertility; the appearance of anthropogenic soil compaction phenomena, through the use of mechanical aggregates during wet periods; mineral nutrition imbalances, which generate high sensitivity to pathogens (diseases, pests, frost, drought, etc.) (Abad et al., 2021).

The soil is the foundation of life, and the quantity and quality of production depend on its health. The annual or permanent vegetal cover, achieved by grassing the space between plant rows, constitutes one of the most important and practical measures for improving soil structure and organic matter, and for conserving and enhancing soil biodiversity (Zehetner et al., 2015).

Long-term grassing can constitute an 'ecological buffer,' which shelters and feeds the beneficial fauna in vineyards (predatory mites from the family Phytoseiidae, wasps from the family Trichogramma) (López-Piñeiro et al., 2013; Likar et al., 2017; Irvin et al., 2016).

Different soil maintenance systems, each with their advantages and disadvantages, generates in time differentiated effects on the soil, the plant and the environment, in the production of grapes obtained and, in their quality, and sanogenity (Kliewer, 2005).

Studies conducted by various researchers have highlighted different values for the foliar area/yield ratio depending on the variety (Iacono, 1994; Murisier, 1996). For red wines, under the conditions of the Midi-Pyrenees viticultural zone, the optimal value of this ratio was 2 m^2/kg (Dufourcq et al., 2002). The quantity and quality of grapes depend on the productivity of photosynthesis, which in turn depends on the foliar area.

MATERIALS AND METHODS

In order to establish the singular effect of the factors under study on the vegetative development of the grapevine, the yields obtained, and their quality, the experimental design was organized in a plot with the Cabernet Sauvignon variety, following the model of monofactorial experiments arranged in randomized blocks.

The experimental factor under study was the soil maintenance system, with its gradations:

a_1 - black field (Mt); a_2 - permanent grass with herbs from spontaneous flora; a_3 - lawn (40% *Lolium perenne*, 33% *Festuca rubra* and 27% *Poa pratensis*); a_4 - phacelia; a_5 - white dwarf clover and lawn (40% *Lolium perenne*, 33% *Festuca rubra* and 27% *Poa pratensis*); a_6 - vetch and lawn (40% *Lolium perenne*, 33% *Festuca rubra* and 27% *Poa pratensis*). All these soil maintenance systems aim to replace the currently practiced soil maintenance system, namely the black field, which consists of performing autumn and spring plowing, five mechanical inter-row hoeings and four manual row hoeings.

The analyzed viticultural climate parameters were: average, minimum and maximum air temperature, precipitation, solar radiation and relative humidity. For the collection of biological material, what represents the reserve of beneficial entomofauna in vineyards, Barber traps were used which capture both beneficial and harmful fauna. The determination and systematic classification of the collected species allows the evaluation of beneficial entomofauna and the appreciation of its role in the vineyard ecosystem, as a natural factor limiting the development of certain pest species.

Grapes were harvested at technological and phenological maturity and analyzed for soluble sugar content and total acidity. The influence of foliar area size on the quantity and quality of grape production was established.

The following analyses were performed: sugar content - determined by refractometry (OIV, 2021a); total acidity - determined by titration with NaOH (OIV, 2021b), gluco-acidimetric index = sugar ratio/total acidity (expressed in g/l tartaric acid); vegetative biomass of the vines hub (foliar area).

RESULTS AND DISCUSSIONS

From a climatic perspective, the year 2024, overall, can be considered a year with high heliothermal resources against a backdrop of low water resources, especially during the early-ripening and maturation phase of grapes. A specific characteristic of the year 2024 was the very high temperatures recorded during the summer months, both in the air, where they frequently exceeded 35ºC and even 40ºC, and at the soil surface, where temperatures of 50ºC and even over 60ºC were frequently recorded. Also, the time interval during which these temperatures were present often exceeded 20-26 days. The high thermal regime, which was accompanied by high values of actual insolation, ensured a normal development of the grapevine plants and good grape ripening (Table 1).

Table 1. The 2024 climatic parameters regarding average, minimum, and maximum temperatures

Month	Average temperature, °C	Minimum temperature, °C	Maximum temperature, °C
January	0.9	-11.2	16
February	7	-5.2	20.4
March	8.3	-1.8	27.5
April	15.2	1.9	31
May	16.8	6.6	29.5
June	25.5	13.7	37.7
July	26.9	14.2	39.5
August	26	14.5	36.6
September	19.7	8.2	35.1

The above-average thermal regime throughout the summer months did, however, lead to a decreased air hygroscopicity of 67.5% (Table 2).

Table 2. The 2024 climatic parameters regarding precipitation, air hygroscopicity, and sunshine duration

Month	Rainfall, mm	Higroscopicity, %	Real sunshine hours
January	59.9	81.8	151.8
February	16.8	77.4	163.1
March	48.6	75.8	215.8
April	63.9	66.1	273.1
May	47.1	64.1	274.9
June	29.5	56.4	339.4
July	54	55.4	344.9
August	23.3	52	333.9
September	86.7	65.4	286.2

The precipitation regime observed during the January to September period was markedly distinct from that of the preceding year, exhibiting a deficit in annual totals (304.0 mm),

yet displaying variations throughout the vine's growing season.
The lowest precipitation levels were recorded in the summer months (May, June, July, and August), resulting in a reduction of the soil's immediate water reserves. The insufficient soil water reserves, in conjunction with the exceptionally high air temperatures, led to the widespread manifestation of grape shriveling in vineyard plantations during September.
The severe decrease in soil humidity, manifested since the early-ripening period, it positively influenced sugar accumulation in the grapes, especially when factoring in the above-average thermal regime. A negative impact on grape production was observed, attributed to the phenomenon of berry withering, which arose from the loss of a greater quantity of water through transpiration than was replenished by soil water absorption.
Soil management systems induced differentiated effects on grape yields, with the highest grape production being obtained in the case of the soil management system with phacelia and vetch with lawn, 3.212 kg/vine hub, also 3.084 kg/vine hub. The lowest grape production was recorded when soil maintenance was carried out through permanent grass with herbs from spontaneous flora and lawn, 2.100-2.120 kg/vine hub, (Table 3).

Table 3. Influence of soil maintenance systems on Cabernet Sauvignon grape production

Graduation	Grape production kg/vine	The average weight of a grape g
a_1 - black field	2.160	93.12
a_2 - permanent grass with herbs from spontaneous flora	2.100	88.64
a_3 - lawn	2.120	92.58
a_4 - phacelia	3.212***	97.84
a_5 - white dwarf clover and lawn	3.028**	93.64
a_6 - vetch and lawn	3.084**	96.68

The analysis of production differences determined by soil management systems indicates that, in comparison with the control (black field), all other soil management systems induced statistically significant positive production differences (distinct and highly significant). The largest production differences were recorded in the case of soil maintenance through phacelia seeding (+1,052 kg/vine hub) and through seeding with vetch and lawn, and white dwarf clover and lawn (+0.924 kg/vine hub), and +0.868 kg/vine hub, respectively.
Regarding the average weight of 100 berries, the soil maintenance systems, compared to the control (black field), induced differences of +14.8 g in the case of soil maintenance with phacelia, +4.8 g with the soil maintenance system using vetch and lawn, and only +1.8 g with the soil maintenance system using white dwarf clover and lawn. When soil maintenance was conducted through permanent grass with herbs from spontaneous flora and lawn, the differences compared to the control (black field) were negative, at -11 g and -5.6 g, respectively (Table 4).

Table 4. Influence of soil maintenance systems on the average weight of 100 berry in the Cabernet Sauvignon variety

Graduation	Weight of 100 berry g
a_1 - black field	129.8
a_2 - permanent grass with herbs from spontaneous flora	118.4
a_3 - lawn	124.2
a_4 - phacelia	144.6
a_5 - white dwarf clover and lawn	131.6
a_6 - vetch and lawn	134.6

As a primary observation, it results that permanent grassing of inter-row spaces and lawn represent the technological interventions through which the competition exerted by perennial grasses in the utilization of water and mineral substances on the grapevine is most prominently manifested. An extended period of drought, combined with an elevated temperature regime and air hygroscopicity below 40%, during the phase of rapid shoot and grape development, resulted in a sharp decrease in photosynthesis. Under these conditions, shoot growth was significantly reduced (by more than 50% in sensitive varieties), a pronounced weakening of the vines was observed, and an early cessation of berry growth occurred (berry weight, in most varieties, is 40-50% lower).
Regarding the sugar content of the grapes, the same trend is observed as in the case of grape production, with the soil maintenance system

using phacelia and vetch with lawn showing 276.7 g/l sugars and 266.1 g/l sugars, respectively. In the case of soil maintenance through permanent grass with herbs from spontaneous flora and lawn, the sugar content decreased by 1.3 to 4.7% (Table 5).

Table 5. Influence of soil maintenance systems on Cabernet Sauvignon grape quality

Graduation	Sugar content, g/l	Total acidity, g/l tartaric acid
a_1 - black field	245.9	3.46
a_2 - permanent grass with herbs from spontaneous flora	234.3	3.59
a_3 - lawn	242.8	3.52
a_4 - phacelia	276.7	2.88
a_5 - white dwarf clover and lawn	252.3	3.38
a_6 - vetch and lawn	266.1	3.32

Changes occurring in the viticultural climate have led to an increase in sugar accumulation in grapes, against the backdrop of a drastic reduction in total acidity. This caused the glucoacidimetric index to take on high values, ranging from 65.26 (soil maintenance system with permanent grass with herbs from spontaneous flora) to 96.08 (soil maintenance system with phacelia) (Table 6).

Table 6. Influence of soil maintenance systems on Cabernet Sauvignon grape quality

Graduation	Gluco-acidimetric index
a_1 - black field	71.07
a_2 - permanent grass with herbs from spontaneous flora	65.26
a_3 - lawn	68.98
a_4 - phacelia	96.08
a_5 - white dwarf clover and lawn	74.64
a_6 - vetch and lawn	80.15

The values obtained are far above those optimal for producing typical and high-quality wines. The must acidity decreased slightly depending on the soil maintenance systems, in parallel with a slight increase in sugar accumulation in the grapes.

The specific climatic conditions of 2024 led to good sugar accumulation in grapes and a reduction in total acidity, regardless of the soil maintenance system. The size of the foliar area influences vegetative growth, grape production, sugar accumulation in berries, as well as other 'noble' compounds (anthocyanins, aromas, etc.). Recent research has emphasized the importance of the foliar area required to produce one gram of grapes (cm^2/g) and the optimal values of this indicator, compatible with maximum sugar accumulation in berries.

For the Cabernet Sauvignon variety, the soil maintenance option using phacelia sowing allows a foliar area of 3,548 m^2/vine hub to support the maturation of 3.212 kg of grapes, while a foliar area of 3,329 m^2/vine hub facilitates the maturation of 3.084 kg of grapes with the vetch and lawn seeding soil maintenance method (Table 7).

Table 7. Influence of soil maintenance systems on Cabernet Sauvignon grape production

Graduation	Foliar area square meters	Grape production kg/vine hub
a_1 - black field	2.142	2.160
a_2 - permanent grass with herbs from spontaneous flora	2.026	2.100
a_3 - lawn	2.094	2.120
a_4 - phacelia	3.548	3.212
a_5 - white dwarf clover and lawn	3.147	3.028
a_6 - vetch and lawn	3.329	3.084

The foliar area required to produce one gram of grapes, under conditions of maximum sugar accumulation at harvest, ranged from 11.05 cm^2/g grape (soil maintenance variant through phacelia) at a sugar content of 276.7 g/l must, to 10.79 cm^2/g grape (soil maintenance variant through vetch and lawn) at a sugar content of 266.1 g/l must (Table 8).

The foliar area required to produce one gram of grapes in the version maintained as a black field is 9.92 cm^2/g of grapes at a sugar content of 245.9 g/l of must.

From Tables 9 and 10, containing the analysis of samples from Barber traps, the situation of soil arthropod fauna can be observed.

Table 8. Influence of soil maintenance systems on the foliar area/production relationship of the Cabernet Sauvignon variety

Graduation	Area required for ripening one gram of fruit (square centimeter/g)
a_1 - black field	9.92
a_2 - permanent grass with herbs from spontaneous flora	9.65
a_3 - lawn	9.88
a_4 - phacelia	11.05
a_5 - white dwarf clover and lawn	10.39
a_6-vetch and lawn	10.79

The foliar area required to produce one gram of grapes in the version maintained as a black field is 9.92 cm^2/g of grapes at a sugar content of 245.9 g/l of must.

From Tables 9 and 10, containing the analysis of samples from Barber traps, the situation of soil arthropod fauna can be observed.

It can be observed that in the case of soil maintenance through phacelia, white dwarf clover and lawn, as well as in the variant with vetch and lawn, the beneficial soil entomofauna was improved, compared to the variants maintained through black field, permanent grass with herbs from spontaneous flora, and lawn variant.

Table 9. The situation of numerical abundance harmful soil arthropod fauna

	14.07.2024						20.09.2024					
	a_1	a_2	a_3	a_4	a_5	a_6	a_1	a_2	a_3	a_4	a_5	a_6
1. Ord. ORTHOPTERA	**1**	**1**	**1**					**2**				
Fam. Tettigoniidae	1	1										
Fam. Catantopidae			1					1				
Fam. Gryllidae								1				
2. Ord. HOMOPTERA	**1**	**7**	**2**	**1**	**2**	**2**	**1**	**3**	**2**		**1**	**1**
Fam. Iassidae		2		1	1			2				
Fam. Cercopidae									1			
Fam. Cicadellidae		5			1	1	1	1	1		1	1
Fam. Aphididae	1		2			1						
3. Ord. LEPIDOPTERA Fam. Geometridae												
TOTAL	**2**	**8**	**3**	**1**	**2**	**2**	**1**	**5**	**2**		**1**	**1**

Table 10. The situation regarding numerical abundance of useful soil arthropod fauna

	14.07.2024						20.09.2024					
	a_1	a_2	a_3	a_4	a_5	a_6	a_1	a_2	a_3	a_4	a_5	a_6
1. Ord. ARANEAE				**2**			**10**	**3**	**2**	**2**		**2**
2. Ord. DERMAPTERA Fam. Forficulidae *Forficula auricularia* L										2	2	3
3. Ord. HETEROPTERA Fam. Nabidae												
4. Ord. COLEOPTERA	**2**				**5**	**5**	**4**		**2**		**2**	
Fam. Carabidae	2				3	3	4				2	
Blitophaga undata Mull.							2					
Calosoma auropunctata L.					2	2						
Harpalus pubescens L.	2				1	1	2				2	
Fam. Staphylinidae									2			
Fam. Cerambycidae					2	2						
5. Ord. DIPTERA Fam. Sciaridae												
6. Ord. HYMENOPTERA		**1**	**1**	**4**	**1**	**1**		**1**	**4**	**38**	**17**	**18**
Suprafam. Chalcidoidea			1	1						2		
Suprafam. Ichneumonoidea												
Suprafam. Formicoidea		1		3	1	1		1	4	36	17	18
TOTAL	**2**	**1**	**1**	**6**	**6**	**6**	**14**	**4**	**8**	**40**	**21**	**23**

CONCLUSIONS

Current grapevine cultivation technologies practiced in viticultural ecosystems are oriented towards the maximum efficiency use of technological inputs in order to achieve sustainable grape productions in terms of quantity and quality, while ensuring durable protection of viticultural ecosystems.

The largest production differences were recorded in the case of soil maintenance through phacelia (+1,052 kg/vine hub) and through seeding with vetch and lawn, and white dwarf clover and lawn (+0.924 kg/vine), and +0.868 kg/vine hub, respectively.

Regarding the sugar content of the grapes, the same trend is observed as in the case of grape production, with the soil maintenance system using phacelia and vetch with lawn showing 276.7 g/l sugars and 266.1 g/l sugars, respectively. In the case of soil maintenance through permanent grass with herbs from spontaneous flora and lawn, the sugar content decreased by 1.3 to 4.7%.

Compared to the control (black field), the variant with soil maintenance through phacelia, to ensure the ripening of a grape production of 3.212 kg, achieved a foliar area of 3,548 m²/vine hub. Under the conditions of 2024, in variants V5-white dwarf clover + lawn (40% *Lolium perenne*, 33% *Festuca rubra*, and 27% *Poa pratensis*), and V6-vetch + lawn (40% *Lolium perenne*, 33% *Festuca rubra*, and 27% *Poa pratensis*), a significant increase in beneficial arthropod fauna was observed, both underground and aboveground. A numerical abundance of beneficial arthropod fauna was recorded, especially those of the orders Coleoptera and Hymenoptera. The variant cultivated with phacelia had a positive effect on increasing the pollinator population.

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THE INFLUENCE OF THE ROOTSTOCK PARTNER FROM THE GRAFTING COMBINATION ON THE QUANTITY AND QUALITY OF GRAPE PRODUCTION OF THE FETEASCĂ NEAGRĂ VARIETY IN THE DEALU MARE VINEYARD

Marian ION, Liliana PÎRCĂLABU, Cristian BURLACU

Research and Development Institute for Viticulture and Enology Valea Călugărească,
2 Valea Mantei Street, Valea Călugărească, Prahova, Romania

Corresponding author email: alexandrinalster@gmail.com

Abstract

The rootstock exerts an important influence on the quality of the grapes, affecting the level of production through the vigor given to the vinifera variety. The research was carried out between 2020 and 2024, in a plantation with the Feteasca neagra variety grafted on rootstocks: 125AA, 2C, RG, 71C, SO4-4, 5BB, 140 Ru, 57D, 26C. Compared to the average (2020-2023), grape production in 2024 decreased by +1.117 kg/vine in the case of SO4-4 rootstock and by 0.464 kg/vine in the RG rootstock. The multiple comparison between rootstocks indicates that the 57D, 71C and 2C rootstocks achieved statistically assured positive production differences compared to the 125AA, 26C and RG rootstocks. Due to the accentuated hydric deficit and a high heliothermic regime characteristic of 2024, the concentration of the sugars varied between 262.9 g/l tartric acid at rootstock 57 D and 241.7 in the case of rootstocks 2C, RG, 26 C and SO4-4, to the detriment of the total acidity with variations between 3.9 g/l tartaric acid (57 D) and 5.7 g/l tartaric acid (125 AA and 2 C).

Key words*: extractible anthocyanins; grafting; polyphenols;* r*ootstocks; total anthocyanins.*

INTRODUCTION

Grafting is an essential practice in viticulture, significantly influencing the growth, yield and quality of grape production. The choice of rootstock plays a crucial role in determining the vine's vigor, resistance to environmental stress and the accumulation of essential compounds in the grapes. This study aims to evaluate the impact of different rootstocks on the quantity and quality of grape production of the Fetească Neagră variety, analyzing the physiological mechanisms by which the rootstock influences the absorption of water and nutrients, the changes on the vigor of the stump and its adaptability to the conditions of biotic and abiotic stress.

The research was conducted over several growing seasons, looking at key parameters such as vine yield, sugar content, total acidity, polyphenol levels, and anthocyanin accumulation. Climatic data from the experimental period were also taken into account to understand the influence of the interaction between the vine and the rootstock.

The results indicate that the selection of the rootstock significantly affects both the quantity and quality of the production of grapes of the Feteasca neagra variety. Rootstocks with high vigor favor higher production, but can negatively influence the accumulation of phenolic compounds and uniform ripening of grapes.

On the other hand, rootstocks with moderate vigor ensure a balanced development of the plants, optimizing the synthesis of anthocyanins and polyphenols. Certain rootstocks have contributed to the increase of production, while maintaining a balanced ratio between sugars and acidity, essential for obtaining superior quality wines.

Other rootstocks influenced the phenolic composition, increasing the concentration of anthocyanins and polyphenols, compounds essential for the coloring intensity and stability of the wine.

Previous studies have shown that the interaction between the variety and rootstock can significantly influence the accumulation of sugars, the acidity level and, finally, the aromatic and taste profile of the wine.

Recently, research has focused on evaluating the long-term effects of different rootstocks on the adaptability of Fetească neagră to climate change, highlighting rootstocks that confer better resistance to drought and thermal fluctuations (Vasilescu et al., 2022). Also, the importance of selecting a suitable rootstock is amplified by the new European regulations aimed at the sustainability of viticulture practices, requiring greater efficiency in the management of water and soil resources (EU Regulation 2019/1009).

The statistical analysis confirmed significant differences between the studied combinations, highlighting the importance of rootstock selection according to winemaking objectives and regional climatic conditions. Also, recent studies show that rootstocks influence the content of aromatic precursors of grapes, having a direct impact on the organoleptic profile of the final wine (Ollat et al., 2016). The study suggests that rootstocks adapted to drought and high temperatures help stabilize grape production and maintain phenolic integrity, being preferred in the context of climate change.

In addition, the interaction of the rootstock with the grafted variety can also influence its behavior in terms of resistance to diseases and pests, as well as its ability to adapt to different types of soil. Recent studies suggest that certain rootstocks can reduce water stress by improving water absorption efficiency, playing an essential role in maintaining the plant's water balance (Keller, 2020).

The metabolic analysis of the grapes from the grafting combinations studied indicates that rootstocks can influence the biosynthesis of aromatic compounds, thus contributing to the definition of the sensory profile of the resulting wine. For example, it has been found that rootstocks with moderate vigor favor the accumulation of aromatic precursors, while more vigorous rootstocks can negatively influence the expression of fine aromatic characteristics of the wine (Santesteban et al., 2018).

This study provides valuable information for winegrowers and oenologists in the selection of the most suitable rootstocks for Fetească neagră, aiming to balance the efficiency of production with the maintenance of the superior quality of the grapes.

MATERIALS AND METHODS

The research was carried out in the ecopedoclimatic conditions of the Valea Călugărească wine center between 2020 and 2024 and the impact of climatic variables (average air temperature, precipitation, relative humidity and duration of sunshine) on grape production and its quality in the Fetească neagră variety grafted on a wide range of rootstocks with a very different genetic origin was analyzed. Rootstocks belonging to the species Vitis riparia (RG) as well as hybrids Berlandieri x Riparia (8B, 5BB, 2C, SO4-4, 125 AA, 57D, 26C, 71C), Berlandieri x Rupestis (140 Ru) and Vinifera x Berlandieri (41 B) were followed.

Within the experimental device, the grafting combinations related to the Fetească neagră variety were placed in the field in randomized blocks, with 5 replications, planting distance of 2.2 x 1.2 m (3787 vines/ha), and the training form is the bilateral cordon spurred on the semi-stem (h=60 cm).

The soil in the experimental polygon is reddish brown molic vertic, with a loam clay texture, weak acid pH (6.1), well supplied with humus (2.2%) and useful mineral elements (N, P, K).

Assessments were made on the grape harvest from a quantitative and qualitative point of view at the technological maturity, at the same stage for all the grafting combinations. The following analyses were performed: sugar content-determined by refractometry (OIV 2021a); total acidity- determined by titration with NaOH (OIV 2021b), The polyphenol content was determined by the Folin-Ciocalteu method (Ribéreau-Gayon et al., 2006), total and extractable anthocyanins were analyzed by spectrophotometry at 520 nm. Regarding the statistical analysis of the data, the means were compared using the ANOVA test to identify significant differences between rootstocks, principal component analysis (PCA) to highlight the relationships between variables, Pearson correlations to determine the associations between production, sugars, acidity and polyphenols, linear regressions to analyze the determinants of grape yield and quality, cluster analysis to group rootstocks according to their performance on Fetească neagră.

RESULTS AND DISCUSSIONS

The meteorological data recorded in the 2020-2024 period indicate a thermal regime characterized by average annual temperatures that oscillate between 11.8°C (2021) and 13.8°C (2024).
Compared with normal (1990-2019), the average temperature during the vegetation period increased by 0.4°C (2022), 0.8°C (2023), respectively 2.2°C (2024) (Figure 1).
The precipitations level was very low in the winter months, with 1.4 mm (2020) and 5.8 mm (2022) recorded in January compared with the multi-year average, 37.7 mm.

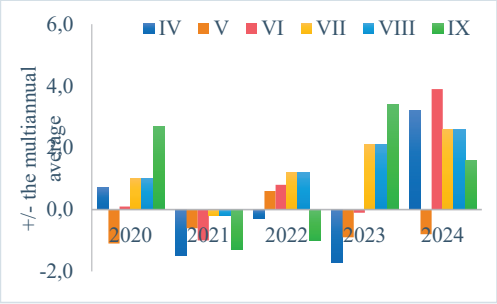


Figure 1. Monthly average temperature during the growing season of the years 2020-2024 compared to the multiannual average of 1990-2019

This became an excess in May when 84.5 mm was recorded compared with 71.6 mm, the multi-year average (Figure 2).

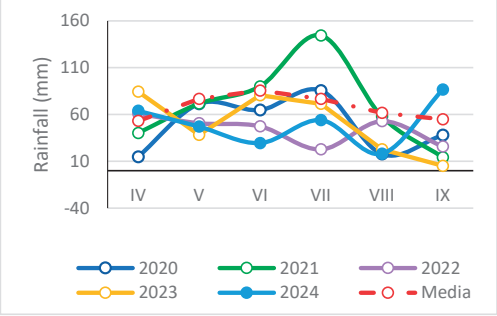


Figure 2. Monthly average rainfall during the growing season of the years 2019-2023 compared to the multiannual average of 1990-2019

The very high air temperatures and the low volume of rainfall in the summer months have made the phenomenon of grape wilting frequently manifest itself in the grape plantations during the veraison-ripening period.
Compared to the 1990-2019 average, the duration of sunshine increased by 21 hours (2021), 34 hours (2020), 47 hours (2022), 275 hours (2023), respectively 304 hours (2024), (Table 1).

Table 1. The duration of sunshine in the period 2020-2024, compared to the average for 1990-2019

Year	Sunshine duration hours
1990-2019	1608
2020	1642
2021	1629
2022	1655
2023	1883
2024	1912

The average amplitude of the production variation in the period 1990-2019 was 0.80 kg/stump, the average grape production showed a minimum in the case of rootstock 93 RG (1.28 kg/vine) and a maximum in the case of rootstock SO 4-4 (2.08 kg/vine), and in the period 2020-2024 the average grape production showed a minimum in the case of rootstock 125 AA (0.74 kg/vine) and a maximum in the case of rootstock SO 4-4 (0.96 kg/vine).
In the Fetească neagră variety, the influence of rootstocks on grape production was obvious. The rootstocks from the Berlandieri x Riparia group had a different behavior.
The SO 4-4 rootstock (0.96 kg/vine) clearly stood out from the other rootstocks, followed by 140 Ru (0.96 kg/vine), 71 C (0.86 kg/vine) and 2 C (0.82 kg/vine), and the 125 AA rootstock (0.74 kg/vine) induced the lowest level of production.
In terms of grape production (kg/vine), the SO4-4 and 140 Ru rootstocks are significantly different from the 125 AA and 2 C rootstocks, while the 2 C and RG rootstocks do not show significant differences (Table 2).
The concentration of the must in sugars, the main qualitative factor of the production, depended to a lesser extent on the rootstocks used in the grafting combinations, with values between 240.4 g/l (140 Ru) and 263 g/l (57 D and Kober 5 BB), (Table 3).

Table 2. The grape production (kg/vine) of the variety/rootstock combinations

Rootstock	Average 1990-2019	Average 2020-2024
125 AA	1.640	0.744
2 C	1.850	0.819
RG	1.280	0.816
71 C	1.940	0.855
SO4-4	2.080**	0.963**
Kober 5 BB	1.760	0.882
140 Ru	1.790**	0.960**
57 D	1.980	0.850
26 C	1.550	0.812

The results obtained highlight the fact that regardless of the climatic conditions during the experimentation period, all the rootstocks taken in the study induced to the vinifera varieties, a level of sugar concentration sufficient to obtain wines with controlled designation of origin.
Rootstock 57 D has the highest sugar content and is significantly different from 140 Ru and 125 AA, and rootstocks K 5 BB and SO4-4 are close in values and do not differ significantly from other rootstocks in the same category (Table 3).

Table 3. The sugar content (g/l) of the variety/rootstock combinations

Rootstock	Average 1990-2019	Average 2020-2024
125 AA	219	245.9
2 C	210	241.7
RG	222	241.7
71 C	221	243.8
SO4-4	222	241.7
Kober 5 BB	221	252.3**
140 Ru	209	240.4
57 D	212	262.9**
26 C	214	241.7

The 57 D, Kober 5 BB rootstocks are significantly different from the RG, 2 C, SO4-4 and 71 C rootstocks, which do not show significant differences (Table 4).
As in the case of the concentration of the must in sugars, the rootstock has a relatively small influence on the acidity of the must.
The average values regarding the acidity of the must varied between 4.1 g/l (57 D) and 4.0 g/l (Kober 5 BB, 140 Ru, 26 C).

The relatively small influence of rootstock on the acidity of the must in vinifera varieties was also highlighted by the researches carried out by: Oslobeanu (1966) and Stoian (1999).

Table 4. The total acidity (g/l tartric acid) of the variety/rootstock combinations

Rootstock	Average 1990-2019	Average 2020-2024
125 AA	5.7**	4.3**
2 C	5.7**	4.2**
RG	5.5	3.9
71 C	5.1	4.2**
SO4-4	6.0**	3.9
Kober 5 BB	5.0	4.0
140 Ru	4.5	4.0
57 D	3.9	4.1
26 C	5.0	4.0

The content in total anthocyanins ranged from 2202 mg/l (125 AA), 2100 mg/l (RG), 2049 mg/l (SO4-4), compared to 2C whose content in total anthocyanins was only 1154 mg/l. (Table 5).

Table 5. The phenolic maturity of the analyzed varieties in the period 2020-2024

Rootstock	Total anthocyanins	Extractible anthocyanins	Extractability
125 AA	2202	1571	71
2 C	1154	783	68
RG	2100	1698	81
71 C	1685	948	56
SO4-4	2049	1366	67
Kober 5 BB	1525	932	61
140 Ru	1725	1101	64
57 D	1756	1195	68
26 C	1762	1195	68

As far as the extractable anthocyanin content is concerned, the variations were between 1689 mg/l (RG), 1571 mg/l (125 AA), 1366 mg/l (SO4-4).
Rootstocks such as 2C, Kober 5 BB, 71 C showed extractable anthocyanin contents of 783 mg/l, 932 mg/l and 71 C, respectively.
Polyphenols influence the structure of wine and its ageing capacity.
The results show that the rootstocks RG, SO4-4 and 125 AA have the highest values of total

polyphenols, indicating a high tannic potential (Table 6).

Table 6. The total polyphenols and the seed maturity of the analyzed varieties in the period 2020-2024

Varieties of grape	Total polyphenols
125 AA	73
2 C	42
RG	99
71 C	49
SO4-4	92
Kober 5 BB	52
140 Ru	57
57 D	69
26 C	61

The significant differences confirm the variability of anthocyanin and polyphenol content between varieties, with less impact on extractability.

In order to better understand how rootstocks are grouped according to production, sugars, acidity and phenolic maturity and to reduce the data sizes and show which variables best explain the variation, principal component analysis was performed.

PC1 (64.2% of the variation) is influenced in Principal Component 1 (PC1) explains 64.2% of the total variation. It is strongly influenced by production and sugars.

Main Component 2 (PC2) explains 25.8% of the variation, being more sensitive to variations in acidity.

Depending on the results obtained, the rootstocks are grouped as follows:

- rootstocks such as 140 Ru and SO4-4 are positioned towards the right end of PC1, which indicates high production.
- 57D and K5BB are grouped towards the left side of PC1 and top on PC2, indicating a high sugar content and moderate acidity.
- the rootstocks in the center of the graph, such as the RG and 71C, provide a balance between production and quality.

This analysis confirms that the selection of the rootstock significantly influences the qualitative and phenolic parameters of Feteasca neagră grapes, and its optimal choice can improve the quality of the wine produced.

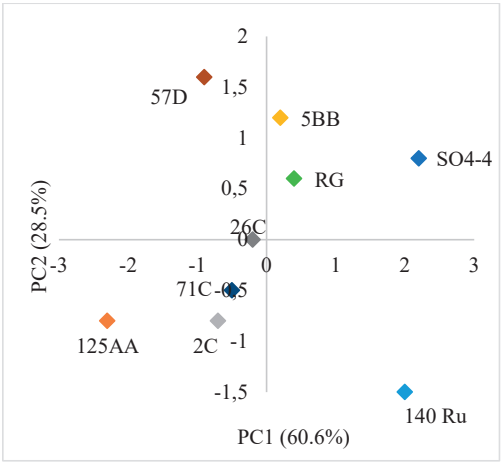


Figure 3. Principal component analyses

CONCLUSIONS

Because the p-value < 0.05, there are statistically significant differences between the analyzed rootstocks in terms of production. In other words, the rootstock significantly influences grape production. Because the p-value < 0.05, there are statistically significant differences between the analyzed rootstocks in terms of sugar content. Therefore, the rootstock significantly influences the sugar content of the grapes. In the case of acidity, the p-value > 0.05, so there are no statistically significant differences between the analyzed rootstocks. This means that the rootstock does not significantly influence the acidity of the grapes.

Clasificarea portaltoilor în funcție de obiective:

If you want to obtain a maximum production rootstock such as SO4-4 and 140 Ru.

If the quality of the production is desired, implicitly a high sugar content, respectively 57 D and K 5 BB quality wines.

If a balance between production and quality is desired rootstocks such as 71 C and RG are recommended.

As for the estimated profit, the most profitable rootstock is 57 D, although the production is not the highest, the high sugar content leads to a higher price per kg and, implicitly, to the highest profit.

The only balanced rootstock is K5BB, which ensures the best combination of production and sugars, generating solid profit.

To maximize production, but moderate profit, SO4-4 and 140 Ru rootstocks are recommended, which ensure high production, but lower sugars limit the price per kg, reducing profit compared to other rootstocks.

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INVESTIGATION OF THE NDVI CORRELATION IN A *VITIS VINIFERA* CV. MERLOT VINEYARD GROWN WITH DIFFERENT NUTRIENT REGIMES UNDER NON-IRRIGATED CONDITIONS

Hristo KOZAROV, Zhulieta ARNAUDOVA, Boyan STALEV

Agricultural University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: hristo.kozarov1998@gmail.com

Abstract

The production of high-quality wine grapes represents a significant challenge for winemakers, both in the short term and on an annual basis. In the context of modern viticulture, the means to achieve this goal include the application of vegetation indices derived from remote sensing.
The objective of this study was to establish a correlation between NDVI and the growth stages of Vitis vinifera cv Merlot vines grown in the village of Brestnik. The study period was the 2023-2024 growing season.
The NDVI values were calculated from time series of Sentinel-2 images for the main phenological stages. The results indicated the vegetative and reproductive potential of the vines and relation with NDVI. The resulting mathematical model and correlations can be used as predictive models.

Key words*: NDVI, grapevine, remote sensing, vineyards, vegetation indices.*

INTRODUCTION

In modern agriculture, optimal vineyard management is particularly important given the impacts of climate change and limited water resources.
Satellite imagery provides a wide range of opportunities for rapid monitoring of agricultural fields (Szabó et al., 2016; van Dessel et al., 2008; Lóki-Szabó, 2011; Burai et al., 2014; Varga et al., 2015). Precision agriculture has received considerable attention in the agricultural community over the last two decades (Sun et al., 2019; Palalottino et al., 2018; Comba et al., 2016). Modern agriculture is characterised by the need for precision vineyard management, especially in the context of global warming and changing climatic conditions. These factors are altering traditional growth phases, with serious implications for grape yield and quality, with traditional vineyard regions adapting to new conditions or even moving to more northern regions (Nistor et al., 2020; Sun et al., 2011; White R., 2009; Popova, 2022).
In viticulture, the precision agriculture approach aims to ultimately improve grape yield and quality in vineyards while reducing the impact of climate change by addressing difficulties in the production cycle through appropriate crop management decisions (Arnó, et al., 2009; Silvestroni et al., 2019).
NDVI is widely used in remote sensing because it provides a quick and efficient method of assessing plant vigour and health, which is particularly important in viticulture. Under non-irrigated conditions, where water resources are limited, effective nutrient management becomes even more critical to achieve optimum grape yield and quality. In this context, the present study aims to analyse the relationship between NDVI and grapevine growth performance, taking into account the influence of different nutrient regimes (Sun, 2017).
Remote sensing using satellite imagery has become an indispensable tool for monitoring crop conditions. Among the many vegetation indices available, the Normalised Difference Vegetation Index (NDVI) stands out for its ability to reflect plant vigour and provide a quick and efficient assessment of plant health. The use of NDVI in viticulture is of particular importance as it allows the tracking of spatial variability in vineyard areas, which is key for the assessment of yield and production quality (Szabó et al., 2016; Burai et al., 2014).
A number of studies have attempted to analyse the relationship between NDVI and agrobiological performance in vineyards grown with different nutrient regimes under non-

irrigated conditions. Under such conditions, limited water availability and variation in nutrient regimes provide additional challenges to vine growth.

Remote sensing using satellite imagery provides a synoptic, timely and broad-spectrum view of actual viticultural conditions, allowing assessment not only of plant vigour but also of key biophysical parameters such as leaf area index (LAI), which are important for yield prediction (Szabó et al., 2016; Lóki-Szabó, 2011).

The results of this study can provide valuable information for the development of agronomic strategies aimed at improving the productivity and sustainability of vineyards in a changing climatic environment.

The aim of this study was to establish a correlation between NDVI and growth stages of *Vitis vinifera* cv Merlot vines grown under different nutrient regimes in non-irrigated conditions in the village of Brestnik.

MATERIALS AND METHODS

The experiment was conducted in the training experimental field of the Agricultural University in the town of Kuklen, which is situated in the Plovdiv district in Central-South Bulgaria (Figure 1).



Figure 1. Location of study area
(Google Earth 23.05.2021)
L42° 3'22.80"N B 24°47'1.19"E

Experimental design and treatments

Satellite imagery

NDVI was obtained from Copernicus Browser. https://browser.dataspace.copernicus.eu/

The data were available for the Area of Interest (AOI) from Sentinel 2 L2A with HR multispectral satellite imagery. The images were obtained for the main growth stages during the investigated period 2023-2024.

Vegetation health assessment is a crucial part of environmental monitoring, precision agriculture, and land cover classification. One of the most widely used vegetation indices in remote sensing and GIS applications is the Normalized Difference Vegetation Index (NDVI). NDVI is a remote sensing-derived index that measures vegetation health and biomass by analyzing the difference between near-infrared (NIR) and red (RED) reflectance values from satellite or drone imagery. Since healthy vegetation strongly reflects NIR light and absorbs RED light, NDVI helps in distinguishing between healthy and stressed vegetation (https://geoinfotech.ng/).

The formula of the NDVI is

$$NDVI = \frac{NIR - RED}{NIR + RED}$$

where:
NIR is near-infrared light;
RED is red light.
For Sentinel-2, the index looks like this:

$$NDVI = \frac{B8 - B4}{B8 + B4},$$

where:
B8 = 842 nm;
B4 = 665 nm.

Using the NDVI data in the study regions, the changes in vegetation cover present in the area and the trend in occurrence of agricultural drought can be studied (Sruthi et al., 2015).

The vegetation index was calculated from Sentinel-2 bands images and sample raster values were processed by GRASS in QGIS 3.34. The growth stages are calculated in the Days Of the Year (DOY). The correlations between the studied variables were obtained by regression analysis in Excel Microsoft 365 and were valid within the time range studied.

In situ measurements

The experiment focused on a Merlot variety grafted onto the rootstock Berlandieri x Riparia SO4. The vines were planted in 2011 in the training experimental base of the Department of

Viticulture and Fruit Growing at the Agricultural University, Plovdiv.
The variety is cultivated under non-irrigated conditions.
The planting distance is 3.0 m between the rows and 1.0 m between the vines in each row.
In the experimental plan, the following 7 variants are defined for the foliar treated, the feeding phases are: First inflorescence appearance, start flowering, after mass flowering, “Pea” size and Veraison.
V0 - control (no treatment);
V1 - treatment at a dose of 200 ml/ha;
V2 - Treatment at 300 ml/ha;
V3 - 400 ml/ha treatment;
V4 - 500 ml/ha treatment
In both variants with incorporation into the soil of "Humate Rost" as (solid residue) is applied in two doses at the beginning of the growing season.
V5 - root treatment at the beginning of vegetation with a dose of 15 kg/ha;
V6 - Root treatment at the beginning of the vegetation period at a rate of 25 kg/ha.
10 plants are included in each variant.
The climate data for temperature and precipitation has been recorded by the climate station in the plantation during the period April-September.

RESULTS AND DISCUSSIONS

The Merlot grape variety is characterized by its medium-ripening tendency, which typically manifests around mid-September. When cultivated with optimal agrotechnical practices and sufficient loading, the vegetative strength of the vines is notably pronounced.
The experiment was conducted in seven variants, with different levels of foliar and root nutrition with liquid fertiliser "Humat Rost". "Humat Rost” is a fully organic nutrient derived from peat. The liquid concentrate is characterised by a deep, dark brown colour and a relatively high viscosity.
This product is a recent innovation developed in Bulgaria (Agroprom LTD) and is part of the organic family.
The primary source of energy in the soil is plant residues and animal excreta. The water-soluble organic compounds of the soils from which "Humate Rost" is mineralized (degraded) by soil microorganisms.
This organic product eventually releases energy that is used by the vines to stimulate the growth of the vegetative and reproductive organs of the grape variety. (https://humatrost.com/)

Climate data

The start and duration of phenological stages are genotype specific and depend not only on hereditary characteristics but also on agro-ecological conditions. Day length and temperature have a direct impact on the growth stages in general and on the vegetative growth and flowering, which is why the vine crop responds differently to climatic conditions.
To conduct a thorough and scientifically sound study, it is necessary to monitor both quantitative and abiotic factors to analyse the changes in the microclimate of the vineyard under study.
From a practical standpoint, it is imperative to emphasise that in 2024, the precipitation levels during the spring season were markedly below average. This resulted in impediments to sap movement and the development of winter buds. Concurrently, the combination of elevated temperatures with the opening of the roof scales of the buds led to a cessation in further growth. This observation underscores the pivotal role of temperature as a restrictive factor, while underscoring the indispensability of water for sustaining growth. The application of “Humat Rost” at doses of 300 and 400 g at 0.1 h in 2023 had a positive influence on the interphase transition in 2024 due to enhanced plasticity of the canes. To optimise the outcomes, it is recommended that this practice be accompanied by the implementation of early spring irrigation or the deepening of inter-rows in vineyards during the autumn season to a depth of 20 cm. This approach will facilitate enhanced aeration and facilitate effective water movement around the root zone.
Temperatures are systematically organized by the decade and signify the increase in temperature. The year 2024 is evidently distinguished by elevated temperatures during the initial ten days of April, which corresponds to the onset of the growing season (Figure 2).

Table 1. Phenological development of the Merlo cultivar

Year	Budding	First inflorescence appearance	Flowering	Veraison	Maturity	Fall leaf	Vegetation period
2023	8.04	21.04	12.06	10.08	14.09	27.11	233
2024	3.04	7.04	29.05	29.07	19.08	17.10	197

The growth and development of vine plants is influenced by a number of factors, including temperature, but also the availability of soil moisture, which is formed by precipitation during the autumn-winter and winter-spring periods of the year.

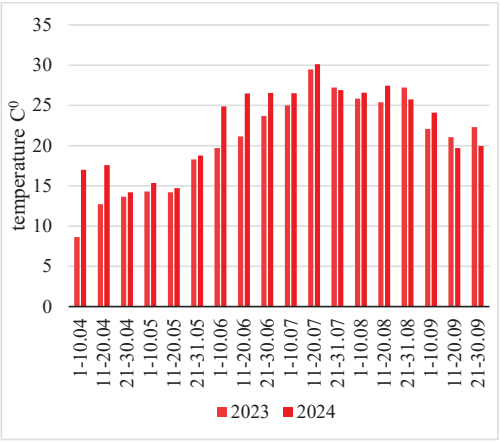


Figure 2. Average temperatures by ten-day periods from April to September

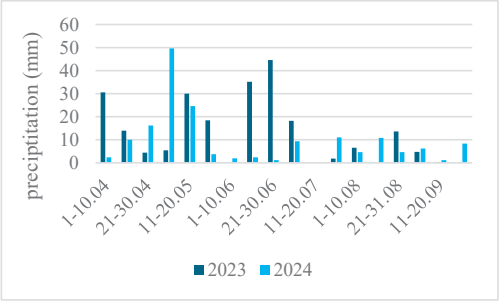


Figure 3. Precipitation totals in mm by ten-day periods from April to September

The spring of 2023 was marked by anomalous seasonal mean temperatures and substantial deviations in precipitation levels.

In particular, April, May and June experienced approximately one-third of the annual mean precipitation typically observed in the Southern wine region.

In 2023, growth processes in the root zone started significantly earlier due to higher temperatures in spring, resulting in a shorter period for mature grapevine pruning.

In the second year of our study, this trend is maintained, with a change in temperature anomalies throughout the 2024 period.

The total precipitation recorded during the entire year of 2023 was 350.4 mm.

The highest recorded precipitation was in June, with 79.8 mm.

The variability in precipitation amounts was significant, with considerable variation observed across the region during the study period (Figure 3).

The combination of elevated summer temperatures and deficient precipitation resulted in the premature termination of growth processes, despite the presence of greater vegetative mass in certain variants.

The utilization of satellite imagery in the initial stages of grapevine development, along with the significance of the vegetation index (NDVI) from the onset of active vegetation (first inflorescence appearance), can provide grape farmers with valuable information to facilitate the implementation of measures that stimulate growth processes and facilitate the transition to subsequent growth stages. The selected cultivar and vineyard are in a transitional-continental zone, where there are periods of irregular precipitation and significant temperature variation. The experimental years had a growing season length of 233 (2023) and 197 (2024) days (see Table 1).

Relationship between NDVI and experimental variants

In the present study, the efficacy of “Humat Rost” growth fertilizer in enhancing foliar and root nutrition was assessed. To this end, regression equations were derived from NDVI values for each of the treatments during the main growth stages and 10 days following the fertilizer application. The temporal progression

of the experiment is delineated by DOY, which denotes days of the year. The results for the 2023 growing season are illustrated in Figures 4(a), 4(b), 4(c), 4(d), while those for the 2024 season are presented in Figures 5(a), 5(b), 5(c), 5(d).

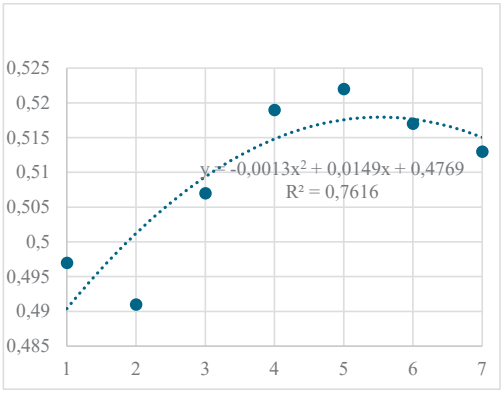


Figure 4(a). Relationship between NDVI and First inflorescence appearance (DOY 110)

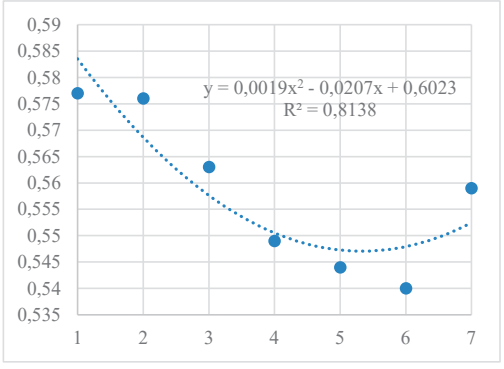


Figure 4 (b). Relationship between NDVI and Flowering (DOY 163)

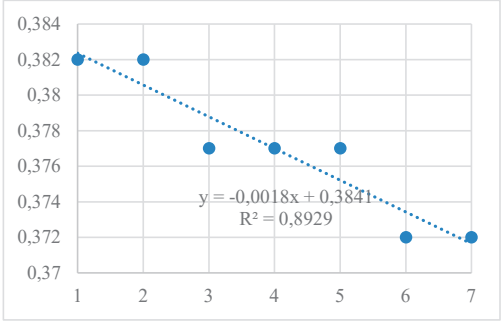


Figure 4(c). Relationship between NDVI and Veraison (DOY 223)

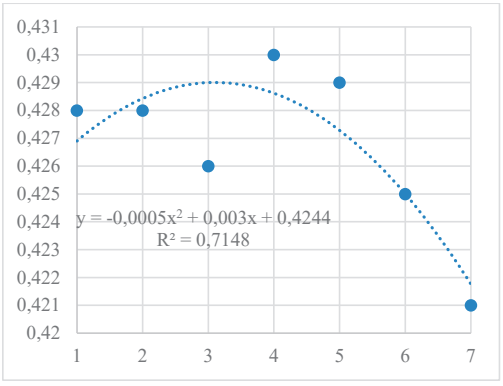


Figure 4(d). Relationship between NDVI and Maturity (DOY 257)

As demonstrated in Figures 4(a), 4(b), 4(c), 4(d) the regression equations derived from the initial research year demonstrate a high multiple correlation coefficient R^2, ranging from 0.71 during Maturity to 0.81 in the Flowering growth phase. These equations are expressed as second-degree curves. With values undergoing a smooth transition during the Version growth stage and are represented by a linear equation.

It is observed that the flowering phase yields high values of NDVI (0.53-0.59), which, at this early stage of plant development, is a very good indicator of plant vigour.

However, as the plants progress through the various stages of development, they are subjected to conditions of drought and elevated temperatures, which results in a decline in NDVI values to 0.37-0.38.

Variants V2 and V3, supplied with the highest concentration of "Humat Rost", exhibited optimal development. Anomalies were observed in the Flowering growth stage, where these two variants demonstrated the lowest values, likely attributable to inadequate nutrient uptake due to elevated temperatures and scarcity of precipitation.

The data for the second year of the study are presented in Figures 5(a), 5(b), 5(c), 5(d), and the resulting regression equations have a high, and very high, multiple correlation coefficient R^2= 0.66-0.97.

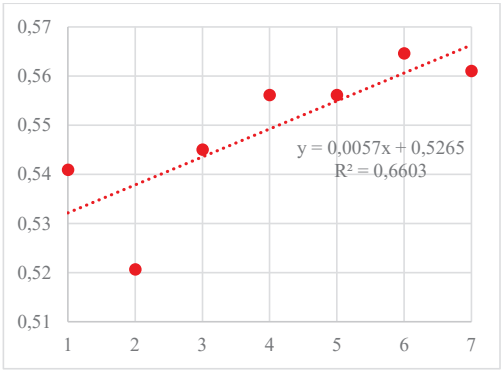


Figure 5(a). Relationship between NDVI and First inflorescence appearance (DOY 100)

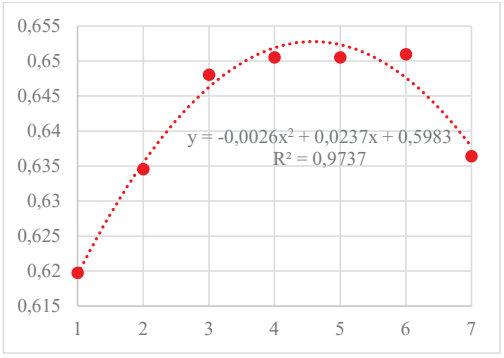


Figure 5(b). Relationship between NDVI and Flowering (DOY 150)

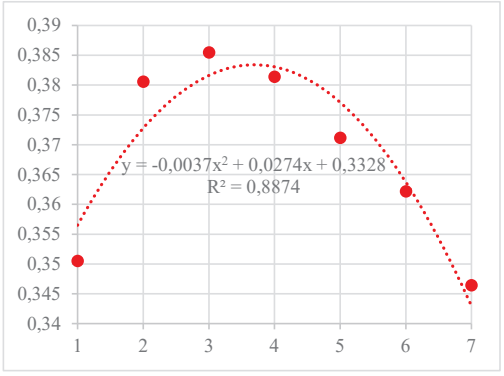


Figure 5(c). Relationship between NDVI and Veraison (DOY 210)

When compared to the results from the previous year (2023), the equations demonstrate a very good representation of the results. The year was distinguished by elevated temperatures and deficient precipitation, particularly during the Veraison and Maturity phenological phases, with NDVI values ranging from 0.3 to 0.39. The optimal development and NDVI values were once again observed for variants V2 and V3, with no aberrations in plant development.
Technical maturity was attained 36 days earlier than the previous year.

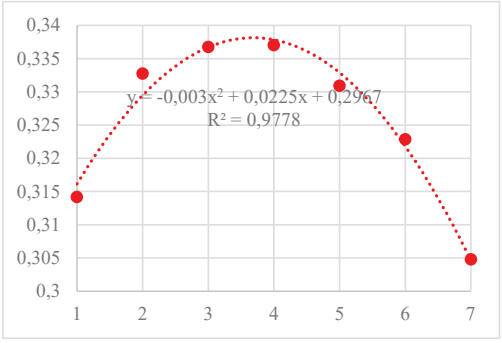


Figure 5(d). Relationship between NDVI and Maturity (DOY 230)

CONCLUSIONS

The present study investigates the efficacy of "Humat Rost" as an organic fertiliser to influence growth, with a focus on its impact. The analysis of the results indicates that the vine crop adapts expeditiously to environmental changes, and depending on the thermal dynamics of spring and the irregular distribution of precipitation in winter and spring, it can concomitantly progress with some of the growth stages.The findings of the research indicate a shortening of the growing season by 233 (2023) and 197 (2024) days, respectively. This finding indicates that, with a discrepancy of approximately 36 days, the vine can effectively compensate for phenological development. on foliar and root nutrition. The experimental setup provides a compelling rationale for recommending the utilisation of the tested organic product as a foliar fertiliser, with doses of 300 and 400 g per 0.1 ha. The regression equations between NDVI and the variants with different nutrient regimes obtained in this study exhibit a remarkably high coefficient of multiple correlation between the individual variations in the studied phenophases, thereby substantiating the in-situ measurements.
The utilisation of satellite imagery and vegetation indices could have facilitated reliable monitoring of vine crops.

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POTASSIUM INFLUENCE ON 'VICTORIA' AND 'PERLA DE CSABA' TABLE GRAPES QUALITY

Eleonora NISTOR[1], Alina DOBREI[1], Dragoş George CONSTANTINESCU[2], Gabriel CIORICA[2], Marcel DANCI[1], Simion ALDA[1], Alin DOBREI[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technologies, 119 Calea Aradului, 300645, Timişoara, Romania
[2]"Victor Babeş" University of Medicine and Pharmacy, 2 Piaţa Eftimie Murgu, 300041, Timișoara, Romania

Corresponding author email: alin1969tmro@yahoo.com

Abstract

The study was conducted between 2021 and 2023 and evaluated the influence of potassium on the quality of 'Victoria' and 'Perla de Csaba' table grapes. The samples were collected from a private vineyard in Masca, Arad County. Three doses of potassium (120, 130, and 140 kg/ha) were applied at three critical phenological stages (before flowering, pre-veraison, and post-harvest). The results indicated that the different potassium (K) doses significantly ($p \leq 0.05$) influenced the grapes' quality. Notably, the dose of 130 kg (K) had a major impact on varieties, influencing sugar content, aromas, and berry size uniformity. The 140 kg/ha potassium dose increased the average bunch weight and significantly ($p \leq 0.05$) decreased berry acidity, particularly in the 'Perla de Csaba' variety. The study highlights the importance of balanced potassium fertilisation to improve key grape quality parameters, such as sugar content and bunch weight. The results suggest that adjusting potassium doses according to grape variety may optimise grape quality outcomes.

Key words: *blooming, harvest, potassium, table grape, veraison,*

INTRODUCTION

A vital component of many physiological and biochemical functions, potassium (K) is necessary for the vigorous growth and development of grapevine (Marcuzzo et al., 2021). Along with nitrogen and phosphorus, potassium is one of the three main macronutrients. It has a direct impact on the quantity and quality of grapes produced as well as the plantation's ability to manage biotic and abiotic challenges (Ciotta et al., 2021).

Balanced fertilisation, according to the specific requirements of the soil and climatic conditions, can considerably increase grape yield and quality while ensuring the crop's long-term viability (Villette et al., 2020). This mobile nutrient helps regulate osmotic pressure in plant cells, promoting water uptake and maintaining turgor pressure. It also regulates stomatal opening, which is essential for transpiration and indirectly influences photosynthesis, affecting vegetative growth and carbohydrate accumulation (Hu et al., 2023).

Applying potassium before bud break promotes root and bud development, preparing the vineyard for rapid growth, flowering, and high-quality grape yields (Schreiner & Osborne, 2020). Table grape varieties have slightly different nutritional requirements compared to wine grape varieties. Potassium plays an essential role in meeting these needs by enhancing the physical characteristics and flavour of the berries (Pushpavathi et al., 2021). This nutrient also aids in the accumulation of energy stores in plants, which is necessary for an appropriate beginning of the growing season (Monteiro et al., 2021).

Potassium promotes bud development and differentiation, contributing to consistent and prolific flowering. This process supports the formation of compact and well-formed clusters, although other factors also play a role (El-Boray et al., 2019).

Potassium regulates ion transport and osmotic balance, indirectly enhancing water and nutrient uptake. This supports efficient and healthy vine growth following budbreak (Eshghi et al., 2011). Potassium facilitates proper water and nutrient absorption, preparing the vines for efficient and healthy growth following budbreak, while also promoting

carbohydrate buildup, which is necessary for grape development (Eshghi et al., 2011).
Applying potassium before budbreak also helps to produce grapes with an appropriate balance of acidity and sugars, which is vital for table grape flavour quality (Rogiers et al., 2017).
Prior to veraison, grapevine potassium requirements increase significantly to support sugar accumulation and final berry development. Adequate potassium application at this stage is critical for optimal nutrition, water consumption efficiency, resistance to abiotic stressors (drought, salinity), and grape quality (Wang et al., 2023). Unlike wine grapes, where potassium primarily influences fermentation and pH balance, proper potassium intake influences major table grape qualities such as skin firmness, colour, and flavour, all of which are important for the clusters commercial appreciation (Zhang et al., 2016). Research on potassium deficiency during the ripening stage indicates that it can reduce grape yield and quality while increasing susceptibility to pathogens and environmental stressors (Sánchez-Mora et al., 2017). Therefore, adequate soil fertility management and timely potassium application are essential for increasing table grape crop efficiency. Applying potassium to table grape varieties after harvest is necessary for preserving vine health and guaranteeing high-quality yields in the next growing seasons (Youssef & Roberto, 2014). Following harvest, the vine continues to build up the nutritional reserves required for future growth. By restocking these reserves, potassium application during this period improves the plant's ability to withstand abiotic stressors and pathogenic infections in the following growing season (Rogiers et al., 2017).
Furthermore, potassium enhances post-harvest durability by supporting cell wall integrity and reducing water loss, which contributes to increased resistance to storage-related decay. Particularly in vulnerable varieties like 'Italia' and 'Afuz Ali', an additional potassium supply might boost tolerance to certain biotic stressors, including fungal pathogens, particularly in vulnerable varieties like 'Italia' and 'Afuz Ali' (Mehmood et al., 2021).
While previous studies have examined the general role of potassium in grapevine development, there is limited research on the optimal potassium doses applied at specific phenological stages for table grape varieties, particularly for 'Perla de Csaba' and 'Victoria'. The purpose of the study was to evaluate the effects of three potassium fertilisation doses (120, 130, and 140 kg/ha) applied at three critical stages of the vegetative cycle: before budbreak (spring), prior veraison (early grape ripening), and after harvest (autumn) on the growth, yield, and quality parameters (such as sugar accumulation, acidity balance, skin firmness, and cluster compactness) of the 'Perla de Csaba' and 'Victoria' table grape varieties. These varieties were selected due to their economic significance and their sensitivity to potassium-driven changes in fruit quality and yield. Also, the study aims to provide information regarding sustainable fertilisation strategies that maximise table grape output and market value by examining potassium's role in critical metabolic and developmental processes.

MATERIALS AND METHODS

Study Area and Soil Characteristics

The Miniș-Masca region of Western Romania is renowned for its vineyards. The climate and soil properties significantly influence grape production quality. The region has a continental climate with notable seasonal fluctuations. Summers are generally warm, while winters can be cold and frosty. Precipitation is moderately distributed throughout the year, with drier summers, creating ideal conditions for viticulture and promoting high-quality grape production.

Soil characteristics

The area is characterized by cambisols with good fertility, suitable for viticulture when properly managed. The topsoil (0-30 cm) consists of loamy sand and sandy loam textures (Table 1), providing efficient drainage and aeration beneficial for vine root systems. Deeper layers (30-60 cm) transition to sandy loam or silt-clay loam, improve moisture retention and support deeper root growth.
The soil pH ranges from 6.0 to 7.0 (slightly acidic to neutral), while calcium, magnesium, and iron concentrations are moderate to high, influencing soil fertility.

Table 1. Characteristics of different soil types in the Masca area

Soil Type	Organic Matter (%)	Total N (%)	Available P (mg/kg)	Available K (mg/kg)	pH	Micronutrients
Cambisols	2.5-4.0	0.2-0.3	10-20	150-200	6.0-7.0	Generally adequate (moderate Fe, Zn, Cu, Mn)
Loamy Sand	1.0-2.0	0.1-0.2	5-10	100-150	6.0-7.0	Typically lower; may require supplementation
Sandy Loam	1.5-3.0	0.15-0.25	10-15	150-200	6.0-7.0	Moderately available; better than loamy sand

The diverse soils from Masca, contributes to the area's aptitude for viticulture. The soils are predominantly well-drained, which is important for grapevine health since it prevents waterlogging and promotes deep root growth. Furthermore, the mineral composition of the soils, influence the flavour profiles of the wines.

Masca area climate (2021-2023)

The distribution of precipitation during the growing seasons (2021, 2022, and 2023) is unbalanced (Figure 1), with certain months receiving more rainfall, raising the risk of fungal diseases, and less moisture, increasing water stress. Precipitation amounts vary substantially over time, with 2021 having both the highest (278.9 mm in January) and lowest (5.5 mm in March) levels. Rainfall changes had an effect on grapevine water supplies.

Each of the three seasons has unique precipitation values or deficits in key months for grapevine development. The wetter April of 2023 promotes fast early growth, whereas the dryer August of 2022 accelerates ripening.

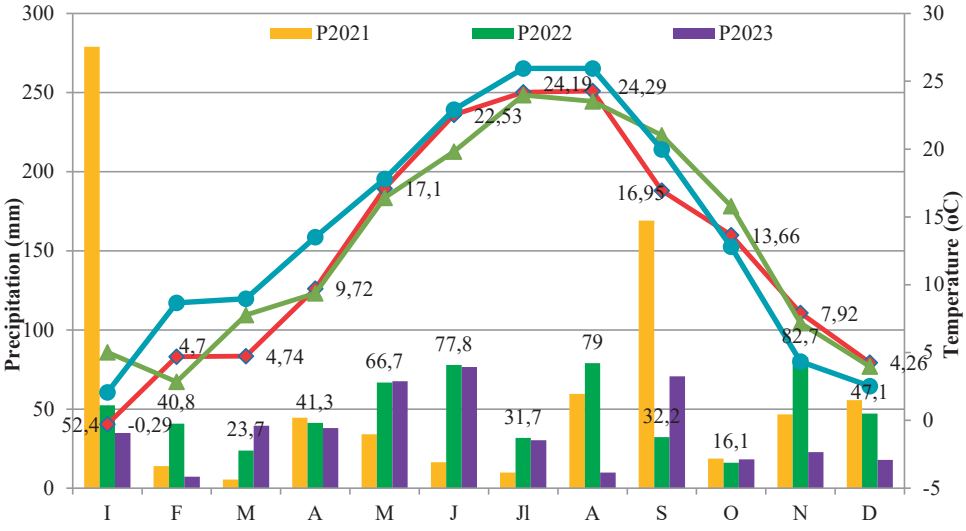


Figure 1. Temperature and precipitation in Masca area during 2021-2023 time

Warmer temperatures accelerated the harvest season (particularly in 2022), necessitating close monitoring to achieve optimal grape ripeness. Lower precipitation in 2023 resulted in smaller berries with more concentrated flavours, which improved wine quality but lowered overall yield. A phenomenon reported during the 2023 grape harvest was a decrease in the number of seeds in the berries, or sometimes their absence, as a result of high temperatures associated with low levels of precipitation.

Experimental Design

A randomized complete block design (RCBD) was implemented to control field variability. The experiment was organised in a factorial design, with two factors: Factor A (potassium dose): 120, 130, and 140 kg/ha; Factor B (application timing): T1 (before flowering - bud break stage, when average daily

temperatures exceeded 10°C), T2 (pre-veraison - 2-3 weeks before the onset of veraison), and T3 (after harvest - immediately after harvest to support reserve building). Each treatment combination was repeated three times. The plot size for each replication was (0.10 ha) to guarantee adequate representation of the vine population. While the main focus was on three potassium (K) doses, an untreated control (without K application) was added to evaluate the relative response. The fertiliser was calibrated to supply 120, 130, and 140 kg K_2O per hectare. Application time for these doses was: before flowering (T1): application was scheduled when the vines reached the bud break stage, typically when average daily temperatures exceeded 10°C and just prior to full bloom; pre-veraison (T2): application was made during the berry development phase, approximately 2–3 weeks before the onset of veraison; post-harvest (T3): application was made immediately after harvest, during the period when vines were building reserves for the following season. Potassium was applied as a granular fertiliser, evenly distributed throughout the allocated plots. In T1 and T2, the fertiliser was gently incorporated into the soil with a shallow cultivator to ensure excellent contact with the root zone. The fertiliser for T3 was spread on the surface since little soil disturbance was desired throughout the post-harvest period. Vines were constantly monitored to determine the timing of phenological stages (bud break, flowering, veraison, and harvest). This ensured that K applications corresponded closely to the specified phenological cycles. All personnel involved in fertilizer application and data collection adhered to standard safety protocols, including the use of personal protective equipment (PPE).

Data collection

Soil samples were taken from the 0-30 cm layer before the study began and after each treatment phase in order to monitor changes in accessible potassium and overall soil fertility.

Plant growth parameters (vine shoot length, leaf area index (LAI), and canopy density were measured on a regular basis); yield components [the number of clusters per vine, cluster weight, and overall grape yield (kg/ha) were recorded at harvest]; and fruit quality (sugar content, acidity, pH, and phenolic content) were analysed on representative samples from each plot. Data were collected at critical growth stages: post-application for T1 and T2 treatments (flowering and veraison), and at harvest (T3) for evaluating final yield and quality.

Plant growth parameters

Vine shoot length was measured manually using a tape measure from the shoot base to the tip of a representative sample of shoots per vine. The leaf area index (LAI) was determined using a ceptometer (LP-80 AccuPAR Ceptometer - Decagon Devices, Inc.) to record photosynthetically active radiation above and below the canopy, and the device's software used the light extinction coefficient to compute the LAI.

Canopy density was measured visually using a standardised canopy rating scale and then analysed using image analysis tools to determine the percentage of ground cover by foliage. When these standardised approaches were used regularly across the vineyard, provide trustworthy data on shoot length, LAI, and canopy density.

During the harvest, a representative sample of vines from each plot was selected to count the number of clusters per vine. Several clusters were randomly selected from both varieties and weighed on a calibrated scale to determine the average cluster weight. Finally, each plot was harvested, and the overall yield was measured and converted to kg/ha according to the plot area. Table grape samples were harvested from representative vines, crushed and pressed to produce juice.

The sugar content was measured with a refractometer (°Brix - C-Tech SAL0025), and the titratable acidity was evaluated by titrating the juice with a standard sodium hydroxide solution and reported as a percentage of tartaric acid. The pH was determined using a calibrated pH meter. The total phenolic content was determined using a spectrophotometric test (Spectrophotometer 6 nm, model V721), with results displayed in gallic acid equivalents.

Statistical Analysis

Statistical analyses were conducted using Excel 2019 (Microsoft Corporation. 2019. Microsoft Excel. Version 2019. Redmond, WA:

Microsoft) and XLSTAT (Addinsoft. 2019. XLSTAT Statistical and Data Analysis Solution. Version 2019. Paris: Addinsoft) to evaluate the effect of different K_2O doses on vine growth, yield, and fruit quality. A one-way and two-way Analysis of Variance (ANOVA) was performed to assess the impact of K_2O application rates on vine shoot length, cluster weight, grape yield, and sugar content, with statistical significance set at $p \leq 0.05$. Additionally, multiple regression analyses were used to determine the relationship between K_2O application and key yield components. Principal Component Analysis (PCA) was applied to identify the primary factors influencing grape yield and quality, summarizing the variance within the dataset and highlighting treatment responses across growing seasons.

RESULTS AND DISCUSSIONS

In this section, the results of the field study for 'Victoria' and 'Perla de Csaba' varieties, are presented over the 2021-2023 growing seasons. Based on Table 2 data, both varieties show an increasing trend in vine growth parameters over the three-year period.

For the 'Victoria' variety, vine shoot length increased from 2021 to 2023, while LAI and canopy density also improved. Similarly, the 'Perla de Csaba' variety exhibited growth with vine shoot length, LAI and canopy density.

Table 2. Growth parameters for 'Victoria' and 'Perla de Csaba' cultivars, for different K_2O doses and application timings, during 2021-2023 growing seasons

Variety	Year	K_2O dose (kg/ha)	Application timing	Vine shoot length (cm)	LAI (m^2/m^2)	Canopy density (%)
Victoria	2021	120	T1	60.1^{b}	3.58^{ab}	75.2^{a}
Victoria	2021	130	T2	61.5^{b}	3.43^{b}	70.5^{b}
Victoria	2021	140	T3	67.4^{a}	3.62^{a}	73.8^{ab}
Perla de Csaba	2021	120	T1	55.2^{c}	3.24^{c}	70.4^{b}
Perla de Csaba	2021	130	T2	57.0^{bc}	3.32^{bc}	69.5^{b}
Perla de Csaba	2021	140	T3	62.5^{a}	3.58^{ab}	72.6^{ab}
Victoria	2022	120	T1	64.0^{b}	3.18^{c}	76.8^{a}
Victoria	2022	130	T2	68.7^{a}	3.35^{b}	78.0^{a}
Victoria	2022	140	T3	66.5^{ab}	3.82^{a}	79.2^{a}
Perla de Csaba	2022	120	T1	58.0^{c}	3.50^{b}	73.5^{b}
Perla de Csaba	2022	130	T2	59.1^{bc}	3.28^{c}	74.8^{ab}
Perla de Csaba	2022	140	T3	61.5^{a}	3.61^{a}	72.3^{b}
Victoria	2023	120	T1	67.0^{b}	4.09^{a}	78.4^{a}
Victoria	2023	130	T2	69.8^{a}	3.90^{ab}	80.0^{a}
Victoria	2023	140	T3	65.4^{b}	4.20^{a}	80.9^{a}
Perla de Csaba	2023	120	T1	59.3^{c}	3.17^{c}	72.7^{b}
Perla de Csaba	2023	130	T2	62.7^{b}	3.35^{b}	73.4^{b}
Perla de Csaba	2023	140	T3	65.3^{a}	3.95^{a}	75.0^{ab}

*LAI - leaf area index; Different letters indicate significant differences at $p \leq 0.05$ within each column; "a" represents the highest statistically significant value; "b" and "c" indicate significantly lower values than "a"; the same letter (e.g., ab), are not significantly different from each other.

'Victoria' consistently exhibited longer vine shoot lengths compared to 'Perla de Csaba' across all years. In general, higher K_2O doses resulted in longer vine shoots, particularly in 2021 and 2022. In 2023, 'Victoria' had its longest shoot length at 130 kg/ha, while 'Perla de Csaba' followed the trend of increasing shoots length with higher K_2O. Statistical differences indicate significant variations, with 140 kg/ha (T3) often leading to significantly longer shoot length. 'Victoria' had higher LAI values compared to 'Perla de Csaba' in most cases, indicating a denser leaf canopy. The highest LAI values were typically observed under the highest K_2O dose, particularly in 2023. In some instances, 130 kg/ha resulted in lower LAI than 120 or 140 kg/ha. 'Victoria' had higher canopy density values compared to 'Perla de Csaba' in all years. The highest canopy density values were recorded in

'Victoria' at 140 kg/ha (T3) in 2023. For 'Perla de Csaba', canopy density was relatively stable across treatments but slightly increased with higher K_2O doses. Unlike vine shoot length and LAI, canopy density was less sensitive to K_2O application timing. These results align with previous research indicating that 'Victoria' berries are larger than those of other varieties, which may contribute to increased shoot vigour. The higher LAI and canopy density observed in 'Victoria' further suggest a more vigorous growth habit, potentially due to its genetic predisposition (Hill et al. 2006). Higher K_2O doses generally resulted in longer vine shoots and increased LAI; this effect can be attributed to potassium's role in promoting photosynthesis and protein synthesis, thereby enhancing vegetative growth (Neve, 2012). The relatively stable canopy density across different K_2O treatments, especially in 'Perla de Csaba', indicates that while potassium influences shoot length and leaf area, its effect on canopy density might be less pronounced (Mpelasoka et al., 2003). This could be due to canopy density being more influenced by factors such as pruning practices and overall vine architecture (Boonterm et al., 2013). The data suggest that K_2O application timing (T1, T2, T3) had a less significant impact on the measured growth parameters compared to the dosage. This finding implies that ensuring an adequate potassium supply is more critical than the specific timing of application (Avenant et al., 1997).

Throughout the three growing seasons, the 'Victoria' variety consistently outperformed the 'Perla de Csaba' cultivar in every yield component (Table 3).

Table 3. Yield components for 'Victoria' and 'Perla de Csaba' cultivars, under different K_2O doses (120, 130, and 140 kg/ha) applied at different timings (T1, T2, and T3) during the 2021-2023 growing seasons

Variety	Year	K_2O dose (kg/ha)	Application timing	Clusters per vine	Cluster weight (g)	Grape yield (kg/ha)
Victoria	2021	120	T1	8.0[a]	150[a]	10,000[a]
Victoria	2021	130	T2	8.4[a]	155[a]	10,200[a]
Victoria	2021	140	T3	8.9[a]	160[a]	10,400[a]
Perla de Csaba	2021	120	T1	7.1[b]	140[b]	9,500[b]
Perla de Csaba	2021	130	T2	7.5[b]	145[b]	9,700[b]
Perla de Csaba	2021	140	T3	7.8[b]	150[b]	9,900[b]
Victoria	2022	120	T1	9.0[a]	165[a]	11,000[a]
Victoria	2022	130	T2	9.5[a]	170[a]	11,100[a]
Victoria	2022	140	T3	10.1[a]	175[a]	11,400[a]
Perla de Csaba	2022	120	T1	8.2[b]	155[b]	9,100[b]
Perla de Csaba	2022	130	T2	8.5[b]	160[b]	9,200[b]
Perla de Csaba	2022	140	T3	9.3[b]	165[b]	9,600[b]
Victoria	2023	120	T1	10.0[a]	160[a]	10,300[a]
Victoria	2023	130	T2	10.5[a]	165[a]	10,700[a]
Victoria	2023	140	T3	11.0[a]	170[a]	10,900[a]
Perla de Csaba	2023	120	T1	8.9[b]	150[b]	10,000[b]
Perla de Csaba	2023	130	T2	9.5[b]	155[b]	10,300[b]
Perla de Csaba	2023	140	T3	10.1[b]	160[b]	10,500[b]

Different letters indicate significant differences at $p \leq 0.05$ within each column; "a" represents the highest statistically significant value; "b" indicate significantly lower values than "a"; the same letter (e.g., ab), are not significantly different from each other.

In 2021, 'Victoria' had all significantly higher ($p \leq 0.05$) yield parameters than the respective values for 'Perla de Csaba'. This trend continued in 2022 and 2023, with 'Victoria' exhibiting a steady increase in clusters per vine, cluster weight and grape yield. In contrast, 'Perla de Csaba' maintained lower values. These statistically significant differences ($p \leq 0.05$) indicate that the 'Victoria' variety is more vigorous and productive under the given conditions, which suggests that genetic factors or better adaptation to the growing environment may be contributing to its superior performance.

Increasing K_2O doses led to a higher number of clusters per vine, with the highest values

observed under 140 kg/ha (T3). The increase in cluster number was more noticeable in 2022 and 2023, suggesting a cumulative effect of K fertilization or favourable environmental conditions. ‘Perla de Csaba’ also followed a similar trend but with slightly lower cluster counts compared to ‘Victoria’, indicating a variety-dependent response to K application. Higher K_2O doses led to increased cluster weight in both varieties. The heaviest clusters were recorded at 140 kg/ha (T3), particularly in 2022. ‘Victoria’ had consistently heavier clusters compared to ‘Perla de Csaba’, likely due to its more vigorous growth and greater ability to partition assimilates. The increase in cluster weight from 120 kg/ha to 140 kg/ha was more pronounced in 2022 and 2023, indicating that adequate potassium supply enhances fruit development. Grape yield followed a similar pattern to cluster weight and number, with the highest yields recorded under 140 kg/ha (T3). ‘Victoria’ showed superior yields compared to ‘Perla de Csaba’, reaffirming its higher productivity potential. The largest yield increase due to K fertilization was observed in 2022, suggesting optimal environmental conditions or improved nutrient uptake. In 2023, yield slightly declined despite higher cluster numbers, possibly due to variations in environmental factors such as temperature, precipitation, or nutrient availability. T3 (after harvest) consistently resulted in the highest values for all yield components, suggesting that post-harvest K application supports better nutrient storage and subsequent growth. T2 (pre-veraison) showed moderate improvements, while T1 (before flowering) had the lowest values, possibly due to earlier nutrient uptake limitations. Keller (2015) highlighted that cultivar-specific traits greatly influence vine vigour and yield potential, while Reynolds (2022) noted that efficient resource utilization is a key determinant of higher cluster weight and overall yield. The superior performance of ‘Victoria’ underscores the importance of cultivar selection in viticultural practices and suggests that further research into the genetic and physiological bases of these differences could inform breeding programs aimed at enhancing yield performance (Ferrara et al., 2017). The positive correlation between elevated K_2O doses and the number of clusters per vine aligns with existing literature emphasizing potassium's role in reproductive development (Obenland et al., 2015). Potassium is integral to various physiological processes, including enzyme activation and assimilates translocation, which are crucial for fruit set and development (Orak, 2017). The more noticeable increase in cluster numbers during 2022 and 2023 may be attributed to the accumulation of K reserves in the vine's perennial structures, enhancing reproductive capacity over time (Mpelasoka et al., 2003). Additionally, environmental factors such as optimal rainfall and temperature during these seasons had synergistically amplified the effects of potassium fertilization (Kodur, 2011). The augmentation of cluster weight with higher K_2O applications is consistent with potassium's known influence on grape development (Mpelasoka et al., 2003). Potassium facilitates the translocation of sugars and other metabolites to developing fruits, thereby increasing their size and weight (Conde et al., 2007). The superior cluster weights observed in 'Victoria' compared to 'Perla de Csaba' suggest a varietal difference in the efficiency of K utilization, possibly due to inherent genetic factors influencing growth vigour and assimilate partitioning. The pronounced increase in cluster weight during 2022 and 2023 further indicates that sustained K fertilization, coupled with favourable environmental conditions, can significantly enhance berry development. The overall grape yield, reflecting both the number of clusters and their individual weights, exhibited a positive response to increased K_2O fertilization (Howell, 2001). The highest yields recorded at the 140 kg/ha application rate (T3) corroborates studies highlighting potassium's critical role in maximizing grapevine productivity. The slight decline in yield observed in 2023, despite higher cluster numbers, is attributed to environmental variables such as temperature fluctuations, precipitation patterns, or nutrient availability affecting berry size and quality. The superior performance of post-harvest K applications (T3) across all yield components suggests that this timing is optimal for replenishing the vine's nutrient reserves. Post-harvest is a critical period when vines actively restore carbohydrates and mineral nutrients in

their perennial tissues, which are essential for supporting early shoot growth and fruit development in the subsequent season (Mpelasoka et al., 2003). Applying potassium during this period ensures its availability during key physiological stages, thereby enhancing overall vine health and productivity (Delgado et al., 2004). In contrast, earlier applications (T1 and T2) may not align as effectively with the vine's nutrient uptake patterns, potentially limiting their efficacy. The consistently higher yield components in 'Victoria' compared to 'Perla de Csaba' indicate a varietal-dependent response to K fertilization. This disparity may stem from genetic differences influencing growth habits, nutrient uptake efficiency, and assimilate allocation. 'Victoria' variety consistently exhibits superior fruit quality compared to 'Perla de Csaba' across all three growing seasons (Table 4).

For instance, in 2021, 'Victoria' grapes had a sugar content of 18.0°Brix versus 17.0°Brix for 'Perla de Csaba', higher acidity, a lower pH and a higher phenolic content. This trend continues in 2022 and 2023, with 'Victoria's sugar content increasing from 2022 to 2023, while 'Perla de Csaba' consistently remains about 1°Brix lower.

Table 4. Grape berry quality for 'Victoria' and 'Perla de Csaba' cultivars, during 2021-2023 growing seasons

Variety	Year	K_2O dose (kg/ha)	Application timing	Sugar content (°Brix)	Acidity (% TA)	pH	Phenolic content (mg GAE/L)
Victoria	2021	120	T1	18.0^{b}	0.61^{b}	3.35^{b}	448^{b}
Victoria	2021	130	T2	18.9^{a}	0.65^{b}	3.40^{b}	453^{b}
Victoria	2021	140	T3	18.4^{b}	0.68^{a}	3.42^{b}	461^{a}
Perla de Csaba	2021	120	T1	17.3^{c}	0.59^{c}	3.49^{b}	430^{b}
Perla de Csaba	2021	130	T2	17.9^{b}	0.60^{b}	3.51^{b}	429^{b}
Perla de Csaba	2021	140	T3	18.2^{b}	0.67^{a}	3.58^{a}	436^{a}
Victoria	2022	120	T1	18.1^{b}	0.62^{b}	3.40^{b}	459^{b}
Victoria	2022	130	T2	18.6^{b}	0.66^{b}	3.47^{a}	463^{b}
Victoria	2022	140	T3	18.9^{a}	0.69^{a}	3.45^{b}	471^{a}
Perla de Csaba	2022	120	T1	17.5^{c}	0.60^{c}	3.30^{b}	432^{b}
Perla de Csaba	2022	130	T2	17.2^{c}	0.62^{b}	3.52^{a}	439^{a}
Perla de Csaba	2022	140	T3	17.9^{b}	0.68^{a}	3.56^{a}	440^{a}
Victoria	2023	120	T1	18.4^{b}	0.63^{b}	3.44^{b}	452^{b}
Victoria	2023	130	T2	18.9^{a}	0.68^{a}	3.41^{b}	465^{b}
Victoria	2023	140	T3	19.1^{a}	0.66^{a}	3.48^{a}	473^{a}
Perla de Csaba	2023	120	T1	17.9^{b}	0.61^{b}	3.49^{b}	441^{b}
Perla de Csaba	2023	130	T2	18.2^{b}	0.63^{b}	3.54^{a}	455^{a}
Perla de Csaba	2023	140	T3	18.7^{a}	0.67^{a}	3.39^{b}	449^{a}

*Different letters indicate significant differences at $p \leq 0.05$ within each column; "a" represents the highest statistically significant value; "b" and "c" indicate significantly lower values than "a"; the same letter (e.g., ab), are not significantly different from each other.

Similar patterns were observed for acidity, pH, and phenolic content, with 'Victoria' always showing statistically significant ($p \leq 0.05$) higher values for acidity and phenolics, and lower pH values than 'Perla de Csaba'. These results suggest that the 'Victoria' variety produces grapes with a more favourable composition for winemaking and higher overall quality, likely due to better adaptation or inherent genetic advantages under the given environmental conditions.

The higher sugar content in 'Victoria' (Table 4) suggests greater efficiency in carbohydrate accumulation, a trait often linked to genetic factors governing photosynthetic capacity and assimilate partitioning (Keller, 2020). Previous studies have reported that varieties with superior sugar accumulation tend to exhibit higher sink strength, allowing better mobilization of carbohydrates to developing berries (Gutiérrez-Gamboa et al., 2021). This aligns with our findings, as 'Victoria' consistently outperformed 'Perla de Csaba' in °Brix levels, likely due to a more efficient source-sink relationship.

The observed differences in acidity and pH reinforce the role of potassium fertilization and varietal metabolism in regulating berry

composition. Higher acidity and lower pH values in 'Victoria' indicate a greater retention of organic acids, which is desirable for maintaining freshness and microbial stability in table grapes and wines (Brunetto et al., 2019; de Orduña R., 2010).

Grapevines regulate pH through potassium accumulation, which influences the balance between malic and tartaric acids during ripening (Sweetman et al., 2009). The lower pH in 'Victoria' suggests that this variety might have a more efficient acid retention mechanism compared to 'Perla de Csaba', contributing to its superior quality.

Phenolic compounds, which impact colour, texture, and antioxidant properties, were significantly higher in 'Victoria' across all seasons. This aligns with recent findings that certain grape varieties possess an inherent advantage in phenolic biosynthesis, which can be further enhanced through optimal potassium nutrition (Hu et al., 2023; Sperling et al., 2024). The higher phenolic content in 'Victoria' suggests a stronger activation of the phenylpropanoid pathway, which is essential for flavonoid and tannin synthesis (Delgado et al., 2004).

Studies indicate that potassium plays a critical role in secondary metabolite accumulation, which enhances both nutritional value and sensory attributes (Hu et al., 2023). Furthermore, the year-to-year increase in sugar and phenolic content in 'Victoria', particularly in 2023, may be attributed to the cumulative effects of sustained potassium fertilization and favourable environmental conditions (van Leeuwen et al., 2019).

Previous research has shown that multi-season nutrient applications lead to improved carbohydrate and secondary metabolite accumulation, as perennial tissues store reserves that contribute to fruit development in subsequent seasons (Nistor et al., 2022). This effect was more pronounced in 'Victoria', further indicating its superior ability to utilize potassium for growth and quality enhancement.

The PCA biplot (Figure 2) provides valuable insights into the relationships between the 'Victoria' (V) and 'Perla de Csaba' (PC) table grape varieties, their responses to different potassium (K_2O) treatments (T1 - 120 kg/ha, T2 - 130 kg/ha, T3 - 140 kg/ha), and the key variables influencing their growth and fruit quality.

The first two principal components, F1 (66.38%) and F2 (11.34%), collectively explain 77.72% of the total variance, indicating that the PCA captures a substantial portion of the dataset's variability and provides a reliable representation of the relationships among the variables.

Relationships among variables

Grape yield (Gy), phenolic content (Ph), canopy structure (LAI, Cw, CD, Cv, VSL), and sugar (S) exhibit a positive correlation. This suggests that increased canopy density and larger leaf area contribute to higher grape yields, greater phenolic content, and enhanced sugar accumulation.

Conversely, pH and acidity (A) display distinct associations with the two grape varieties, reflecting their differential responses to potassium treatments.

'Victoria's response to K_2O treatments

'Victoria' (represented by V22T3, V23T2, and V23T3) shows a stronger positive response to increased K_2O application. Specifically, higher potassium levels (T2 and T3) are associated with increased yield, improved canopy vigour, and enhanced fruit quality (phenolic content and sugar).

The positive correlation between 'Victoria' and acidity (A) under higher potassium treatments suggests that this variety maintains a more balanced pH and acidity level, which is favourable for fruit quality. Among the potassium treatments, T3 (140 kg/ha) appears to have the most pronounced effect on improving yield and quality traits in 'Victoria'.

'Perla de Csaba's response to K_2O treatments

'Perla de Csaba' (represented by PC21T1, PC22T1, and PC23T2) exhibits a more scattered distribution in the PCA biplot and is positioned away from the high-yield variables. This indicates a weaker response to K_2O application regarding productivity. Interestingly, pH is more strongly associated with 'Perla de Csaba' (PC21T3, PC22T3), indicating that this variety tends to maintain a higher pH and lower acidity compared to 'Victoria'.

Despite increased potassium doses, 'Perla de Csaba' does not show a significant positive correlation with key productivity and quality

traits, suggesting that its response to fertilization is more limited.

These findings provide a clear understanding of how potassium application influences grape yield and quality, aiding in the optimization of fertilization strategies for different grape varieties.

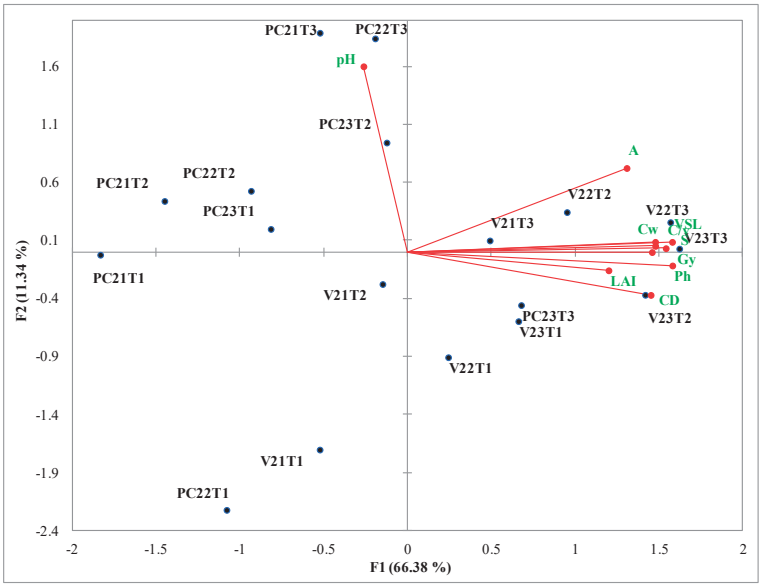


Figure 2. The PCA biplot represents the relationships between variables and treatments (2021-2023): V –'Victoria'; PC – 'Perla de Csaba'; T1 –treatment 120 kg K_2O; T2 – treatment 130 kg K_2O; T3 –treatment 140 kg K_2O; Clusters per vine (C/v), Grape yield (Gy), Vine shoot length (VSL), Cluster weight (Cw), LAI (Leaf area index), Canopy Density (CD), Sugar (S), Acidity (A), Phenolic content (Ph)

CONCLUSIONS

The study demonstrated that 'Victoria' consistently outperformed 'Perla de Csaba' in vine growth, yield, and fruit quality during the 2021–2023 growing seasons. Among the potassium fertilization treatments, the highest dose (140 kg/ha, T3) applied post-harvest resulted in the most significant improvements across all measured parameters, particularly in the 'Victoria' variety. 'Victoria' exhibited greater shoot length, leaf area index (LAI), and canopy density, which contributed to enhanced photosynthetic efficiency and increased yield components such as cluster weight, total grape yield, and the number of clusters per vine. While 'Perla de Csaba' also responded positively to potassium fertilization, its improvements were comparatively modest. Fruit quality parameters, including sugar content, acidity balance, phenolic concentration, and lower pH values, were more pronounced in 'Victoria,' indicating a stronger varietal response to increased potassium availability. Principal Component Analysis (PCA) revealed strong associations between potassium application, vine growth, and productivity, supporting the role of potassium fertilization in optimizing agronomic traits. The study suggests that post-harvest potassium application (T3) is the most effective strategy for enhancing vine growth, yield, and fruit composition during the observed period. However, further research is needed to evaluate the long-term effects of potassium fertilization on soil health, environmental sustainability, and economic viability. Future studies should explore how potassium interacts with other macronutrients, assess the cumulative impact on vine longevity, and investigate the cost-effectiveness of high-dose potassium applications across diverse climatic and soil conditions. These insights will contribute to developing more sustainable and precise potassium fertilization practices for table grape production.

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OPTIMIZATION OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM GRAPE POMACE USING SUPERCRITICAL LIQUID (CO_2) METHODOLOGY

Petronela -Anca ONACHE[1], Irina Elisabeta GEANĂ[2], Corina Teodora CIUCURE[2], Dorin Ioan SUMEDREA[1], Victoria ARTEM[3], Cătălin Marian DUCU[4], Georgiana CÎRSTEA[4]

[1]National Research & Development Institute for Biotechnology in Horticulture Ştefăneşti - Argeş, 37 Bucharest-Piteşti Road, Ştefăneşti - Argeş, Romania
[2]National Research and Development Institute for Cryogenic and Isotopic Technologies, 4 Uzine Street, Râmnicu Vâlcea, Romania
[3]Research and Development Resort for Viticulture and Winemaking Murfatlar, 2 Calea Bucureşti, Murfatlar, Romania
[4]National University of Science and Technology POLITEHNICA Bucharest - Piteşti University Center, 1 Târgul din Vale, Piteşti, Romania

Corresponding author email: dsumedrea@yahoo.com

Abstract

The extraction of phenolic compounds and antioxidant activity (AA) of winemaking by-products using supercritical liquid methodology has been investigated. In order to have a green product, CO_2 was used in extraction. Extraction by supercritical liquids on grape pomace and wine enriched with total polyphenolic compounds (TPC) from extracts and antioxidant activity were evaluated. Several grape varieties from two different vineyards were used Stefanesti and Murfatlar. The extract TPC ranged from GAE/L to 156.66 mg to 2536.89 mg GAE/l, and for fortified wine ranged from 1510 mg GAE/L to 7457 mg GAE/L. Antioxidant activity in fortified wine varied between the values of 54.45 mmol TE/L and 216.13 mmol TE/L. With the help of chromatography several phenolic compounds, such as gallic acid, were identified, shikimic acid, caffeic acid, catechin, epi-catechin, syringic acid, p-coumaric acid

Key words*: Chlorophyll fluorescence water deficit, Vitis vinifera L, environmental drought stress, photosystem II efficiency*

INTRODUCTION

Grapes resulting from the winemaking process lead to the generation of increasing quantities of pomace (Zemni, 2023), reaching about 8.5 million tons worldwide annually (Abreu, 2025). Due to the increasing amounts of waste, the increase in greenhouse gas emissions and the decrease in natural resources (Colnik, 2024), the policy and objectives of protecting the environment in the EU provide for finding measures and ways to transform wine waste into valuable components. Improper disposal of wine-making waste on the ground leads to serious problems of soil contamination, groundwater contamination, and the spread of vine-specific diseases, with risks even to the health of the human population (Abreu, 2025).
The utilization of wine pomace to obtain different compounds responds to the principles of the circular economy, also leading─to a sustainable economy of the wine industry (Abreu, 2025; Liu, 2024; Matos, 2019; Yang, 2009).
Grape pomace contains tannins, polyphenolic acids (hydroxybenzoic and hydroxycinnamic acids), catechins, and other compounds, such as phenolic compounds, anthocyanins, fibers, vitamins, lipids, lignocellulosic compounds and minerals (Abruu, 2025), which can serve in the food, pharmaceutical and cosmetic industry (Abru, 2025; Rodrigues, 2022; Matos, 2019).
Obtaining extracts from pomace with high contents of active compounds and minimal losses, depending on the non-conventional methods and techniques used, the amounts of organic solvents energy consumption processing times, etc. (Khaw, 2017). One of these technologies is the extraction of supercritical CO_2 fluids.

Supercritical fluid extraction (SFE) is an environmentally friendly technology based on reduced processing energy input, alternative use of solvents, the possibility of recovering the gaseous solvent (CO_2) which can be stored in cylinders and reused in future extraction, leading to an increase in the sustainability of the use of supercritical fluid extraction (SFE) technology.

This extraction method, uses little or no organic solvent (ethyl alcohol) and can utilize supercritical fluids in extractions. Due to the variation in density, liquid/gas, viscosity and high diffusion (Khaw, 2017; de Melo, 2014; Fornari, 2012) these supercritical fluids have been successful in their use in extractions, due to their durability (Khaw, 2017).

This study aims to obtain functional ingredients through a greener extraction method (extraction with supercritical CO_2 fluids) and enrich the wine with these bioactive compounds with high nutritional value.

MATERIALS AND METHODS

Raw material. Grape pomace (*Vitis vinifera* var. 'Cabernet Sauvignon', 'Burgund Mare' and 'Merlot') was collected from the winery in INCDBH Stefanesti and from the SCDVV Murfatlar winery ('Mamaia', 'Pinot Noir' and 'Băbească neagră'), the harvest 2023. The marigold samples were frozen and stored before the analysis. Grape pomace was dried at 45 ◦C for 5 minutes at tunnel microwave drying tape JW-MW-6 KW (Onache, 2022). The grape pomace samples before drying had humidity between 85-75%, and after drying they reached between 5-8%, after which the samples were shredded using the mill with Fritsch Pulverisetti 16 hammers.

Optimization process with experimental extraction equipment with supercritical liquids. Supercritical CO_2 extraction of polyphenolic compounds from the 6 varieties of grape pomace was performed on the SFT-110 SFE SYSTEM equipment at the following parameters: the solvent is pharmaceutical ethyl alcohol and the supercritical liquid is CO2; the ratio of supercritical CO2 liquid to pharmaceutical ethyl alcohol is 20:1; Extraction power is 3000 psi; Flow rate: 6 mL/min CO2; Static extraction: 10 Minutes (4 cycles); Dynamic extraction: 10 minutes (4 cycles). 30 G of grape pomace was used and 200 ml of extract was obtained.

Analytical Investigations. Quantitative UV-Vis Spectrophotometric Determinations

Total polyphenols in extract/wine (PFT). The phenolic compounds in wine are oxidized by the Folin-Ciocalteu reagent. In reaction with phenolic compounds, tungsten and molybdenum oxides are formed, which are blue and read at the spectrophotometer at the maximum absorbance with a wavelength of 750 nm (Onache, 2023). The optical density of the gallic acid standard solutions is drawn right by absorbent calibration - concentration and e calculates its parameters, slope and order at the origin.

The result is expressed in mg/l gallic acid and is calculated with the following formula:

$$(mg\ GAE/l)=Dilutie*(A760-b)/a \quad (1)$$

in which: *a* represents the slope of the calibration line; *b* is originally ordered for the calibration line, A760 is absorbance at 760 nm (DO 760 nm).

Determination of Anthocyanins (TA) in extract/wine is carried out with the method of determination by discoloration with SO_2 and is based on the property of anthocyanins to react with SO_2, forming colorless products. Prepare the mixture consisting of: 1 ml of filtered wine, 1 ml of HCl 0.1%, and 20 ml of HCl 2%. From this mixture are prepared two solutions, which are put in 2 test tubes having the following composition: Solution 1 of 5 ml mixture and 2 ml distilled water. Solution 2 of 5 ml mixture and 2 ml of soil 16% sodium metabisulfite.

After 20 minutes, the optical density of solutions 1 and 2 is measured against distilled water, at the wavelength of 520 nm, in the bowl of 1 cm. The result is expressed in mg/l and is calculated with the following formula:

$$TA(mg/l)= 875 * \Delta d \quad (2)$$

where Δd is the difference in optical density of the two solutions.

Determination of Catechins (TC). The method of determining catechins is the reaction with vanillin and is based on the reaction of the phloroglucinol cycle with vanillin, with the production of a red color, stable in concentrated acid solutions. The maximum absorption shall

be recorded at a wavelength of 500 nm. It is read according to the calibration curve at the wavelength of 500 nm.
The result shall be expressed in mg/l catechin and calculated using the following formula:

$$TC\ (mg/l)=Dilutie*(a\Delta d+b) \quad (3)$$

in which: a and b are the parameters of the calibration line Δd is reading to DO 500 nm.

The determination of Tannins (TT) in extracts/wines is the method of determination of tannins (Onache, 2022) is based on the property of tannins to turn hot (100°C) and strongly acidic environment (HCl concentrate) in cyanidin, which is red.
The optical density at the wavelength of 550 nm is directly proportional to the concentration of tannins. Dilute the red wine so that the optical density is in the range of 0.3-0.7. In red wine, the most common dilution is 1/50. Prepare in two test tubes the following solution: 2 ml diluted wine, 1 ml distilled water, 3 ml HCl concentrate.
One of the test tubes is boiled in the water bath at 100°C for 45 minutes. Cool the test tube with tap water. In both test tubes, add 0.5 ml of 96% ethanol to stabilize the color.
It is read at the wavelength of 520 nm, in the bowl of 1 cm, the optical density of the solutions in the two test tubes (boiled and uncooked). The reference is distilled water. Calculation formula:

$$TT\ (mg/l) = \Delta OD\ 520\ x15.7 \quad (4)$$

(ΔOD 520 - the difference between the boiled and unboiled sample, read at the wavelength 520 nm).

Antioxidant activity (AA) was determined in the extract/wine using 3.9 ml of DPPH solution - 1,1-diphenyl-2-picryl hydrazil 25 mg/1,000 ml of methanol together with 0.1 ml of grape pomace extract in methanol solution incubated for 45 minutes at t=25°C, and was also used with trolox (carboxylic acid-2 , 2 , 6-hydrozil 2,5,7,8-tetramethylchrom 0,05 mM in methanol solution used as standard to convert the inhibitory capacity of the solution of methanolic extract from grape pomace into trolox as equivalent or millimoles for antioxidant activity (Tarola A.M et al., 2019).
A spectrophotometer was determined at wavelength 515 nm and the initial absorbance after 45 minutes for each vine variety. The formula for calculation is:

$$AA = \left(1 - \frac{Ac - Aex}{Ac}\right) x\ 100,\ mmol\ TE/100\ ml \quad (5)$$

Polymerised tannins. The V/La ratio is the indicator of the degree of polymerization of tannins. V - represents the optical density of the colored combinations with vanillin, and La - is the optical density of the anthocyanidins formed by heating in acid medium.
The lower the value of the ratio, the higher the degree of polymerization of tannins.

Polyphenolic Profile by UHPLC-HRMS.
The quantitative analysis of individual polyphenols (phenolic acids, flavonoids, and stilbenes) was performed by UHPLC–ESI/HRMS (ultra-high-performance liquid chromatography-electrospray ionization tandem mass spectrometry) using a high-resolution Q Extractive mass spectrometer™ Focus Hybrid Quadrupole OrbiTrap equipped with HESI, coupled to a high-performance liquid chromatograph UltiMate 3000 UHPLC (Thermo Fisher Scientific, City, State, Country).

Chemicals and Reagents. Folin-Ciocalteu phenol reagent (pure) was purchased from Carl ROTH GmbH Co. (Karlsruhe, Germany), radical scavenging assay reagents DPPH (95% 1,1-diphenyl-2-picryhidrazyl) were purchased from Acros Organics (Slovakia), Trolox 97% (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) was obtained from Alfa Aesar (Thermo Fisher GmbH Kandel, 76870 Kandel, Germany), gallic acid and vanillin were purchased from Carl ROTH GmbH Co. (Karlsruhe, Germany), and H_2SO_4 96% and HCl 37% were from Chemical Company (Bucharest, Romania). All chemicals and solvents used in chromatography were obtained from Carl Roth GmbH Co. (Karlsruhe, Germany) and Merck Co. (Darmstadt, Germany), and they were of HPLC or analytical grade (>99%) quality.
Wine Samples. The wine was obtained from the winemaking of the 6 varieties from which the grape pomace resulted, in the same year of production, 2023. The wine was enriched with 0.5% extract from the grape pomace of each variety.

RESULTS AND DISCUSSIONS

Table 1. Description of the varieties from which the extraction was made, the cultivation area, and their abbreviation

Crt No.	Sample of grape varieties	Origin	Extract/ Wine code	Wine+ 0,5% extract code
1	Burgund Mare	INCDBH Ștefănești	BM-St /w	BM-St-SFE
2	Cabernet_Sauvignon		CS-St /w	CS-St-SFE
3	Merlot		M-St_/w	M-St-SFE
4	Băbească neagră	SCDVV Murfatlar	BN-Mf_/w	BN-Mf-SFE
5	Mamaia		MM-Mf_/w	MM-Mf SFE
6	Pinot Noir		PN-Mf_/w	PN-Mf SFE

Figure 1 shows the bioactive compounds obtained from grape pomace extracts (the polyphenol content, anthocyanins) and antioxidant activity. It is noted that the extracts have a very low content of anthocyanins, by fermenting the wine on the breastbone and by the treatments suffered by the grape pomace, most anthocyanins are destroyed.

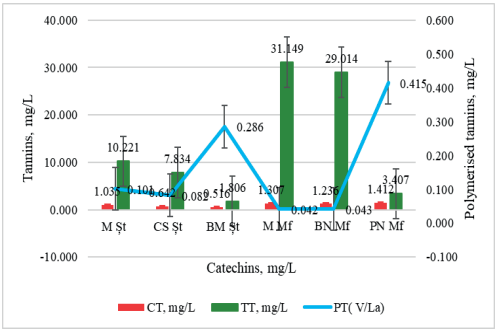


Figure 1. Content of flavonols in extracts obtained by supercritical liquids, *Notes* CT - total catechins, TT- total tannins and PT- polymerized tannins*

The total content of flavonols (abreviere, mg/l) obtained from grape pomace extracts was higher in the 'Mamaia' and 'Băbeacă neagră' varieties cultivated in the Murfatlar geographical area espectively 31.149 mg/l and 29.149 mg/l. At the opposite pole, the lowest concentrations of total flavonols were recorded in the extracts obtained from the 'Burgurnd Mare' variety, cultivated in the Stefanesti wine region. The high concentrations of total flavonols recorded in the extracts obtained from the 'Mamaia' variety led to a decrease in tannins, a decrease in the content of polymerized tannins, and a low content of catechins.

The polyphenolic profile of extracts (Table 2) made by UHPLC-HRMS is shown below.

Table 2 presents the results obtained from grape pomace extracts using supercritical liquid CO2, for all the compounds found. Flavonols are found in fresh grape pomace, but degrade as they undergo treatments, drying and extraction with organic solvents. Catechin is present in three values 0.01mg/L (CS_Sf) - 0.31mg/L (MM_Mf).

Epicatechin had values between 9.04 mg/L in the 'Băbească Neagră' variety and 0.03 in the 'Burgund Mare' variety. Shikimic acid is an important polyphenolic acid in plant synthesis and was highlighted by large quantities in the grape pomace extract. The highest values were recorded in the extracts obtained from the 'Mamaia' variety (9.29 mg/L), cultivated in the Murfatlar region, and 4.49 mg/l as was recorded in the extracts obtained from the 'Merlot' variety, cultivated in the Stefanesti wine region. Phenolic acids are found in high quantities, for example gallic acid, which is found in the grape pomace extract between 0.05mg/L (BM-St) and 15.64 mg/L (MM_M). It has a low content for CS_St (0.09 mg/L) compared to the reference 5.49 mg/100 g DW (Rockenbach, 2011) for the same variety, but it falls with other 'Mamaia' variety, compared to the 'Bordeux' variety in reference (Rockenbach, 2011). Another high phenolic acid is caffeic acid, ranging from 0.03 mg/L (CS_St) to 38.87 mg/L (MM_Mf).

The wine obtained at the two winemaking centers (Stefanesti and Murfatlar) was fortified with 5% extract, obtained by the CO2 supercritical fluid extraction method. The fortification of the wines was done as follows: for one liter of wine, 5 ml of extract obtained by the SEF-CO2 method were used.

Table 2. Individual phenolic compounds of pomace extracts obtained by supercritical liquid CO_2

Polyphenol profiles/grape pomace varieties mg/L	BM-St	CS-St	M-St	BN-Mf	MM-Mf	PN-Mf
shikimic acid	0.87	3.63	4.49	4.40	9.29	3.06
gallic acid	0.05	0.09	0.11	15.64	14.82	2.02
3,4-DHB acid	1.05	2.29	2.66	1.14	3.65	3.00
2.5 DHB acid	n.d.	0.04	0.02	0.05	0.11	0.04
4 HDB acid	0.30	0.31	0.52	0.46	0.60	0.40
caffeic acid	0.06	0.03	0.04	28.70	38.87	0.52
catechins	0.03	0.01	0.02	0.13	0.31	0.00
epicatechins	0.03	0.04	0.03	9.04	8.10	0.15
siringic acid	1.77	7.59	13.56	13.49	19.44	15.03
oenin	n.d.	n.d.	n.d.	4.57	4.42	n.d.
p-cumaric acid	0.09	1.65	2.79	0.40	0.47	0.11
polydatin	n.d.	0.46	0.47	0.31	0.43	0.41
t-resveratrol	n.d.	0.91	0.00	0.81	2.53	n.d.
taxifolin	n.d.	0.01	0.04	0.16	0.34	0.07
suberic acid	2.25	1.47	2.04	0.03	6.34	0.35
vitexin	n.d.	0.03	0.06	0.59	0.42	0.01
hesperidin	0.29	n.d.	0.28	0.42	0.44	0.28
ferulic acid	1.08	n.d.	0.00	0.08	0.65	n.d.
rutin	0.29	n.d.	0.28	0.42	0.44	0.28
plorizin	0.23	0.27	0.33	n.d.	0.47	0.33
elagic acid	0.79	1.04	0.94	0.99	1.90	1.37
azelaic acid	4.41	3.42	6.09	2.01	14.95	2.46
quercetin	0.82	1.07	0.97	1.02	1.93	1.40
abscisic acid	0.01	0.05	0.10	0.26	0.30	0.17
sebacic acid	4.04	0.08	0.32	0.05	1.31	0.08
ploretin	n.d.	0.02	0.02	n.d.	0.07	0.02
kaempferol	0.14	0.14	n.d.	0.56	1.76	0.14
EGCG	n.d.	0.32	n.d.	0.34	0.34	n.d.

n.d. - not determined

The wine obtained at the two wine centers (Stefanesti and Murfatlar) was fortified with 5% extract, obtained by the extraction method of supercritical fluids CO_2. The Figure 2 shows the total polyphenol content in wine, before and after fortification with the extracts obtained.

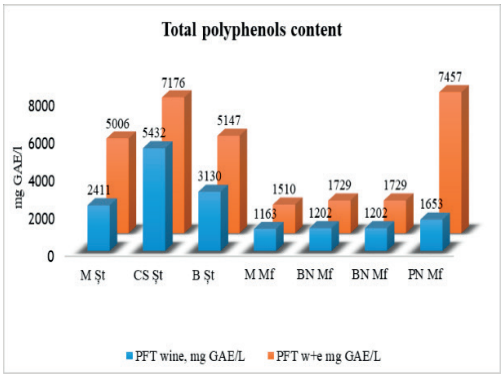


Figure 2. Total polyphenols content of wine varieties before and after fortification with pomace extract biocompounds (**Notes PFT wine - represents total polyphenolic compounds in the initial wine, PFT w+e - represents total polyphenolic compounds in fortified wine with extract*)

In Figure 2, it is noted that the highest amount of total polyphenols, existing in fortified wine, is found in 'PN_Mf' SFE (7457 mg GAE/L), even though the initial wine had a rather low concentration (1653 mg GAE/L). The same difference in terms of total polyphenol concentrations was also evident in SFE 'CS_St' (7176 mg GAE/L).

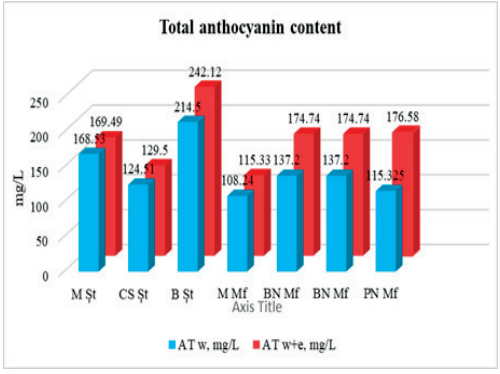


Figure 3. Total content of anthocyanins from wine varieties before and after fortification with biocompounds pomace extracts

By drying the grape pomace are destroyed from anthocyanins (Figure 3), as well as by extraction of SFE-CO_2, for this reason fortified wines do not have very large quantities of anthocyanins. A significant increase is in fortified wine by 'PN_St'_SFE (288.58 mg/l) compared to wine without extract 'PN_St' (254.19 mg/l).

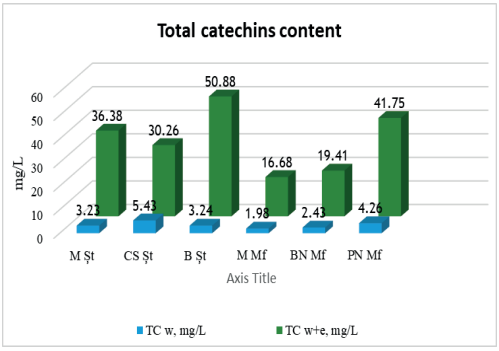


Figure 4. Total content of catechins of wine varieties before and after fortification with pomace extracts

The content of catechins (Figure 4) in the fortified wine was high than in the original wine, it increased with the of addition of grape pomace. Almost all fortified wines have an increase of about 10 times higher than the original wine. The highest concentration is in the 'BM_St' SFE wine (50.8831 mg/l), followed by 'PN_Mf' SFE (41.7521 mg/l).
The tannin content is lower in the fortified wines (Figure 5). 'PN_Mf' SFE (68.61 mg/l) is higher than the original 'PN_Mf' wine (14.0929mg/l).

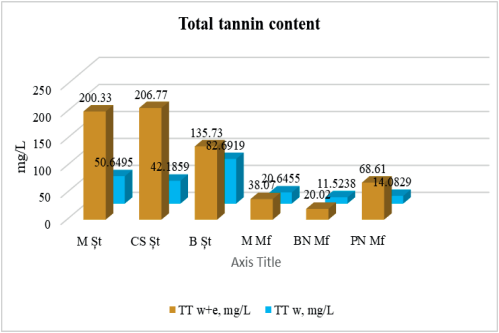


Figure 5. Total content of tannins of wine varieties before and after fortification with pomace extracts

In Figure 6, the antioxidant activities of Stefanesti wines are noted, the highest concentration is observed in fortified wine 'CS_St' SFE (216.13 mmol TE/100 ml), followed by fortified wine 'M_St' SFE (125.98 mmol TE/100 ml). The highest antioxidant activity of a fortified wine from Murfatlar is 'PN_Mf' SFE (101.56 mmol TE/100 ml).

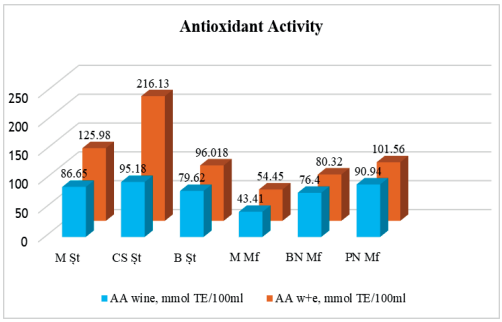


Figure 6. Antioxidant activity for wine varieties before and after fortification with pomace extracts

Table 3 presents the correlations established between the polyphenolic compounds in the original wine and the fortified wine.
A positive correlation was established between the total antioxidant activity of the polyphenolic compounds and the content of catechins
A negative correlation was established between the content of polymerised tannins and tannins (-0.828).
Positive correlations were also established between the antioxidant activity, anthocyanins (0.586) and catechins (0,790).

Table 3. Correlation matrix (Pearson)

Variables	TPF mg GAE/L	TA, mg/L	TC, mg/L	TT, mg/L	PT mg/L	AA, mmol TE/g
TPF mg GAE/L	**1**	0.476	**0.958**	0.504	-0.102	**0.765**
TA, mg/L	0.476	**1**	0.266	-0.130	0.388	**0.586**
TC, mg/L	**0.958**	0.266	**1**	**0.631**	-0.191	**0.790**
TT, mg/L	0.504	-0.130	**0.631**	**1**	**-0.828**	0.390
PT mg/L	-0.102	0.388	-0.191	**-0.828**	**1**	0.170
AA, mmol TE/g	**0.765**	**0.586**	**0.790**	0.390	0.170	**1**

The influence of grape pomace extract on the bio-compound content of fortified wines is outline in Figure 7. The 85.45% PCA score is a very good score.

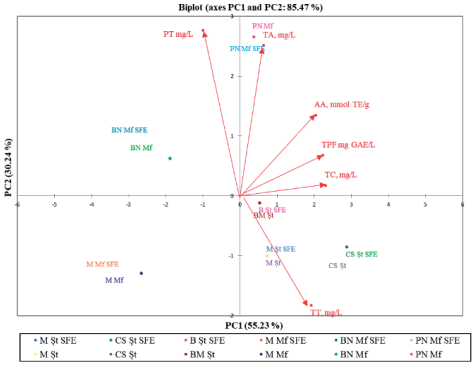


Figure 7. Distribution and principal component analyzed (PCA), according to the composition of polyphenolic compounds in the original and fortified wines

The samples were also analysed with HPLC-HRMS to identify individual polyphenolic compounds in wine and fortified wine.
Table 4 shows the polyphenolic profile of wine varieties and wines fortified with grape pomace extract.

The influence of grape pomace extract on the content of individual polyphenols in fortified wines is underlined in Figure 8.

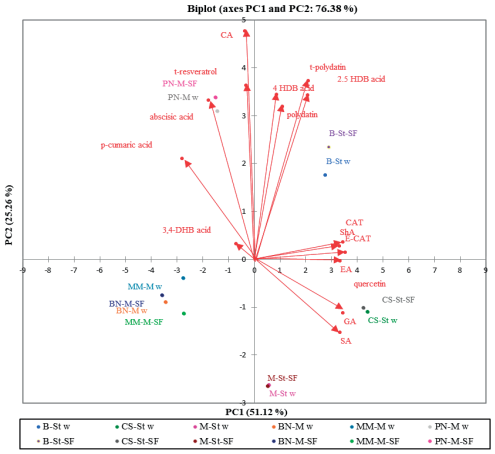


Figure 8. Distribution and principal component analyzed, according to the concentrations of compounds highlighted in the original and fortified wines

Table 4 Individual phenolic compounds of wine and fortified wines

Individual Polyphenols / Varieties	B-St w	CS-St w	M-St w	BN-M w	MM-M w	PN-M w	B-St-SF	CS-St-SF	M-St-SF	BN-M-SF	MM-M-SF	PN-M-SF
ShA	31.69	38.54	30.41	21.46	17.67	27.91	35.18	39.69	31.38	22.96	17.98	27.99
GA	76.55	110.73	67.87	5.78	14.81	14.63	78.39	112.75	65.97	6.52	17.12	15.20
3,4-DHB acid	6.02	4.53	5.85	7.32	4.31	5.02	7.03	4.48	5.79	7.19	4.37	5.30
2.5 HDB acid	1.36	1.02	1.01	0.58	0.72	1.42	1.39	1.33	0.94	0.49	0.66	1.57
4 HDB acid	0.89	0.79	0.44	0.86	0.42	0.92	0.97	0.80	0.47	0.85	0.44	0.85
CA	8.91	4.06	3.70	4.33	6.39	13.63	9.68	3.98	3.42	3.85	6.41	13.85
CAT	50.76	76.05	30.45	10.10	15.68	27.93	51.86	64.91	33.78	9.98	16.41	29.35
E-CAT	19.18	25.56	12.98	1.24	3.11	7.27	19.57	21.33	11.96	1.21	3.23	7.70
SA	250.11	406.84	315.82	33.03	78.85	86.61	253.73	415.50	266.38	34.72	79.26	84.86
p-cumaric acid	2.29	1.18	1.11	4.74	3.02	3.75	2.84	1.37	1.33	5.07	3.24	3.87
polydatin	2.06	0.98	0.75	0.44	1.60	1.23	2.24	0.85	0.76	0.60	1.29	1.32
t-resveratrol	3.03	1.22	0.92	1.08	3.10	1.95	3.11	1.05	0.71	1.72	2.05	2.66
t-polydatin	535.73	494.52	264.85	266.46	219.77	625.74	536.83	474.26	250.31	194.60	180.88	501.23
EA	0.87	0.92	0.37	0.23	0.19	0.15	0.89	0.92	0.44	0.24	0.23	0.20
quercetin	0.87	0.92	0.37	0.23	0.19	0.15	0.89	0.92	0.44	0.24	0.23	0.20
abscisic acid	0.34	0.40	0.30	0.50	0.45	0.71	0.36	0.39	0.32	0.53	0.42	0.78

CONCLUSIONS

The grape pomace has a complex chemical composition, with many biocompounds needed in human nutrition. In this study were determined total polyphenolic compounds and individual bioactive compounds, among which: phenolic acids, stilbene and flavonoids. According to the chromatographic analysis using UHPLC-HRMS, phenolic compounds are individually identified. The main component analysis (PAC) demonstrated that antioxidant

activity may be associated with the phenolic composition of the grape pomace. The extracts from grape pomace showed high antioxidant activity in all grape varieties, the highest is seen in the Pinot Noir variety from Murfatlar (131.606 mmolTE/100 ml). Due to the significant concentration of polyphenolic compounds found in the grape pomace of the Mamaia variety from Murfatlar (1261.11 mg GAE/L), this waste can be reused in the food industry, in functional foods, as in our study was wine. The fortified wine with the highest total polyphenol content is Cabernet Sauvignon (7176 mgGAE/L) and Burgund (5147 mgGAE/L) from Stefanesti, and the wine with the highest antioxidant activity is Cabernet Sauvignon from Stefanesti (216.13 mmolTE/100 ml).

From the study it is noted that the CO_2 supercritical liquid extraction method is a sustainable management method for wine waste. It can be used as a method of fortifying wines. The sanogenic value of fortified wines can be mentioned.

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THE BEHAVIOR OF GRAPE VARIETIES FOR RED WINE IN THE VALEA CALUGAREASCA WINE CENTER

Liliana PÎRCĂLABU, Marian ION, Steliana Paula BARBU, Georgeta TUDOR, Alexandra BUCUR,

Research and Development Institute for Viticulture and Enology Valea Călugărească, 2 Valea Mantei Street, Valea Călugărească, Prahova, Romania

Corresponding author email: stelianap.barbu@gmail.com

Abstract

The researches were carried out in the period 2020-2024, at the wine center Valea Calugareasca within six viticultural plantations with the varieties Feteasca neagra, Cabernet Sauvignon, Merlot, Negru aromat, Novac and Olivia, particularly valuable varieties from an oenological point of view, which are part of the basic assortment for the production of quality red wines. In the last 4 years, the climate has changed in the sense that the thermal regime has increased and the water deficit was accentuated especially during the vegetation period of the vine. The level of the sugars in grape juice has varied between 270.4 g/l (Negru aromat), 254.4 g/l (Novac) and 237 g/l (Merlot), but secondary metabolites such as anthocyanins, negatively affected by high temperatures and with oscillations between 1854 mg/l (Novac), 1540 mg/l (Cabernet Sauvignon) and 1155 mg/l (Olivia). Regarding the content in total polyphenols, the maximum was obtained by the varieties Olivia (81 mg/l), Novac (78 mg/l), Negru aromat (73 mg/l) compared to the variety Cabernet Sauvignon, in which the content in total polyphenols was only 41 mg/l.

Key words*: grape production, sugars; varieties, anthocyanins, total polyphenols.*

INTRODUCTION

Red wines are among the most appreciated types of wines due to their complex sensory characteristics, high content of phenolic compounds, and aging potential. The origin and grape varieties used play an essential role in defining wine quality, influencing its chemical composition, tannin structure, and aromatic intensity (Jackson, 2008).

In recent decades, research in oenology has highlighted the importance of genetic and climatic factors in the accumulation of anthocyanins, polyphenols, and sugars in grapes. These compounds are essential for the color, stability, and balance of red wines (Ribéreau-Gayon et al., 2006). High temperatures and prolonged sun exposure can intensify anthocyanin synthesis, while regions with moderate precipitation favor a balance between sugars and acidity (Matthews & Nuzzo, 2007).

Beyond genetic and climatic influences, viticultural and technological practices play a crucial role in determining the final quality of red wines. Studies show that strategies such as yield regulation, leaf management to optimize sun exposure, and selecting the optimal harvest time can significantly impact the accumulation of secondary metabolites in grapes (Jones & Davis, 2017).

Romania has a significant viticultural diversity, cultivating both indigenous varieties, each with distinct enological potential. Recent studies indicate that varieties such as Fetească neagră, Cabernet Sauvignon, and Merlot have a high content of polyphenols and anthocyanins, giving them great potential for producing high-quality wines (Teodorescu et al., 2011). Moreover, international market trends show an increased preference for wines with high phenolic compound content due to their health benefits and distinctive character (González-SanJosé et al., 2008).

Climate change has a significant impact on vineyards and, consequently, on the quality of red wines. The rise in global temperatures influences vine phenology, leading to earlier ripening periods and affecting the balance between sugars and acidity. Higher temperatures favor sugar accumulation and acidity reduction, which can impact wine structure and aging potential (Jones et al., 2005). Additionally, variations in precipitation can

alter the extractability of phenolic compounds, resulting in significant differences in color intensity and tannin stability (van Leeuwen & Darriet, 2016). Adapting grape varieties to new climatic conditions is essential for maintaining wine quality, requiring the selection of clones resistant to thermal stress and the use of optimized viticultural practices to mitigate the effects of climate change.

This study aims to analyze the compositional differences among various red wine grape varieties, offering insights into their potential and the influence of climatic conditions on wine quality.

MATERIALS AND METHODS

The study aimed to compare and interpret quality characteristics to highlight the potential of each variety in producing high-quality wines.

The studied varieties included Cabernet Sauvignon (CS), Fetească neagră (FN), Merlot (M), Negru aromat (NA), Novac (N), Negru moale (NM), Negru vârtos (NV), and Olivia (OL).

Climate data used in this analysis were collected from the Research Institute for Viticulture and Enology Valea Călugărească station for the period 2020 to 2024. The analyzed viticultural climate parameters included air temperature, precipitation, and solar radiation.

Assessments were made on the grape harvest from both a quantitative and qualitative perspective at technological maturity, at the same stage for all experimental variants.

The following analyses were performed: sugar content, determined by refractometry (OIV, 2021a); total acidity, determined by titration with NaOH (OIV, 2021b); and the glucose-acidimetric index, calculated as the sugar/total acidity ratio (expressed in g/L of tartaric acid).

The analyzed data originate from measurements conducted on grapes from different varieties, evaluating total and extractable anthocyanin content, total polyphenols, extractability, and seed maturity.

The statistical analysis includes mean comparisons, variance analysis (ANOVA), and principal component analysis (PCA) to identify significant differences among varieties.

This study aims to analyze the compositional differences among various red wine grape varieties, offering insights into their potential and the influence of climatic conditions on wine quality.

RESULTS AND DISCUSSIONS

Overall, the years 2020-2024 were characterized by high heliothermal resources combined with low water availability, particularly during the veraison-ripening period of the grapes.

A specific feature of these years was the occurrence of extremely high air temperatures during the summer months, frequently exceeding 35ºC and even reaching 40ºC (Table 1).

Table 1. The climatic parameters of average temperature, precipitation and duration of sunshine in the period 2020-2024

Year	Average temperature °C	Rainfall mm	Sunshine duration hours
2020	15.7	20.1	1642
2021	17.9	18.8	1629
2022	18.5	19.9	1555
2023	19.9	20.2	1883
2024	20.6	21.7	1912

Characteristic for the period 2022-2024 are the very high air temperatures and the low volume of rainfall in the summer months, which caused the grape wilting phenomenon to occur frequently in the grape plantations during the berry ripening-grape ripening period.

The frequency of the occurrence of risk factors ranged between 30% (2022) and 80.6% (2020 and 2024) (Table 2).

Table 2. The frequency of appearance of the risk factor in the period 2020-2024

Year	2020	2021	2022	2023	2024
May	3.2				
June	30.0	23.3	33.3	20.0	86.7
July	61.3	71.0	74.2	84.0	90.3
August	80.6	48.4	74.2	77.0	80.6
September	33.3	3.3	6.7	26.7	36.7

Grape production per vine varied significantly among varieties, indicating differences in individual vine productivity.

Regarding grape yield, variations ranged between 1.956 kg/vine (2024) and 2.364 kg/vine (2021) for the Fetească neagră variety (Table 3).

The Cabernet Sauvignon and Merlot varieties show the highest levels of production, confirming their high production potential, also presenting the highest F-statistical values, which confirms a strong variation in production between years.

Table 3. The grape production (kg/vine) of the analyzed varieties in the period 2020-2024

Year/grape varieties	2020	2021	2022	2023	2024
FN	2.284	2.364	2.264	2.008	1.956
CS	2.986	2.896	2.656	2.684	2.512
M	3.012	2.994	2.856	2.812	2.736
NA	2.554	2.632	2.522	2.128	2.022
N	2.748	2.726	2.638	2.526	2.368
OL	2.689	2.624	2.526	2.312	2.228
NM	2.528	2.432	2.364	2.329	2.167
NV	2.436	2.402	2.361	2.286	2.128

On the other hand, the Novac and Negru moale varieties show moderate variability, but remain significant, indicating a possible impact of climatic factors (Table 4).

Table 4. The statistical significance of grape productions in the period 2020-2024

Grape varieties	F-Statistic	p-Value	Statistical significance
FN	7.85	<0.01	***
CS	9.12	<0.01	***
M	8.45	<0.01	***
NA	5.43	<0.05	**
N	5.62	<0.05	**
OL	6.98	<0.01	***
NM	6.27	<0.05	**
NV	7.14	<0.01	***

$p < 0.01$ (very significant difference)
$p < 0.05$ (significant difference)

The potential for accumulation of sugars in the must, a variety characteristic influenced by the climatic factors during the ripening period of the grapes, was variable from one variety to another.

Thus, the Negru aromat variety had the highest sugar content in 2024 (270.4 g/l), 256.8 g/l (Cabernet Sauvignon), 252.6 g/l (Merlot), 248.2 g/l (Fetească neagră) (Table 5).

The lowest sugar contents were recorded in 2020, respectively 228.9 g/l (Negru aromat), 218.6 g/l (Cabernet Sauvignon), 216.2 g/l (Novac). The sugar content of musts increased with the increase in temperature and with the decrease in grape production.

Table 5. The sugar content of grape must (g/l) of the analyzed varieties in the period 2020-2024

Year/grape varieties	2020	2021	2022	2023	2024
FN	206.8	212.6	224.8	238.6	248.2
CS	218.6	224.8	238.6	242.2	256.8
M	210.4	216.9	234.8	240.6	252.6
NA	228.9	236.4	248.6	256.2	270.4
N	216.2	228.8	238.6	246.2	254.4
OL	212.4	214.6	226.8	232.4	236.4
NM	198.6	206.8	212.4	216.8	224.4
NV	186.8	199.6	206.8	212.6	216.8

The total acidity of the must in the climatic conditions specific to the period 2020-2024 oscillated within very wide limits, with a pronounced variability for this property.

The total acidity showed variations between 3.4 g/l acid tartar (2024) in the Novac variety and 4.1 g/l acid tartaric in the Negru moale variety (Table 6).

Table 6. The total acidity of grape must (g/l tartric acid) of the analyzed varieties in the period 2020-2024

Year/grape varieties	2020	2021	2022	2023	2024
FN	4.6	4.6	4.2	3.9	3.5
CS	4.4	4.2	4	3.8	3.6
M	4.6	4.2	4.2	3.9	3.7
NA	4.9	4.6	4.3	3.8	3.6
N	4.6	4.4	4.0	3.6	3.4
OL	4.6	4.4	4.2	4.0	3.9
NM	5.2	4.8	4.6	4.4	4.1
NV	5.2	4.6	4.4	4.0	3.8

Correlating these data with the climatological analysis, it is observed that high temperatures and longer duration of exposure to the sun favored the accumulation of sugars, while higher rainfall contributed to maintaining a balanced acidity, essential for the freshness of red wines.

In 2024, the gluco-acidimetric index took over very high values, with variations from 75.1 (Negru aromat), 74.8 (Novac), 71.3 (Cabernet Sauvignon) (Table 7).

Phenolic maturity makes it possible to forecast the quality of red wines and to model grape winemaking technologies in the direction of improving the phenolic structure of wines.

The best seed maturity was achieved by the Olivia, Negru aromat and Fetească negră varieties (Table 8).

Table 7. The gluco-acidimetric index of the analyzed varieties in the period 2020-2024

Year/grape varieties	2020	2021	2022	2023	2024
FN	45.0	46.2	53.5	61.2	70.9
CS	49.7	53.5	59.7	63.7	71.3
M	45.7	51.6	55.9	61.7	68.3
NA	46.7	51.4	57.8	67.4	75.1
N	47.0	52.0	59.7	68.4	74.8
OL	46.2	48.8	54.0	58.1	60.6
NM	38.2	43.1	46.2	49.3	54.7
NV	35.9	43.4	47.0	53.2	57.1

Table 8. The phenolic maturity of the analyzed varieties in the period 2020-2024

Varieties of grape	Total anthocyanins	Extractible anthocyanins	Extractability
FN	1130	792	71
CS	1650	765	48
M	1296	794	67
NA	836	685	86
N	2326	1562	59
OL	1242	1086	95
NM	256	156	72
NV	472	264	57

Polyphenols influence the structure of wine and its ageing capacity. The results show that the varieties Negru aromat, Novac and Olivia have the highest values of total polyphenols, indicating a high tannic potential (Table 9).

Table 9. The total polyphenols and the seed maturity of the analyzed varieties in the period 2020-2024

Varieties of grape	Total polyphenols	Seed maturity
FN	45	28
CS	45	28
M	49	34
NA	78	67
N	82	25
OL	87	52
NM	28	81
NV	48	79

The significant differences confirm the variability of anthocyanin and polyphenol content between varieties, with less impact on extractability.

Regarding the content of total anthocyanins, extractable anthocyanins and extractability, the results obtained show significant differences between varieties, some presenting a high content of total anthocyanins (Feteasc㠠neagr㠠and Cabernet Sauvignon), while others have lower values, but with a high extractibility (e.g. Novac, Negru moale). Extractability is a key factor in establishing the coloring intensity of wines. Varieties with high extractibility can generate wines with a more stable and intense color. There is a contrast between the varieties, some having a high extractibility, which suggests an easy extraction of phenolic compounds in the must.

It is known that the maturity of the seeds influences the balance of tannins and the astringent sensation of the wine. Varieties with more mature seeds tend to offer finer tannins, favoring the achievement of a balanced and harmonious wine.

Climatic factors play a key role in the accumulation of anthocyanins and polyphenols, thus influencing the quality of red wines. High temperatures favor the biosynthesis of anthocyanins, but can reduce their extractibility, while excessive rainfall can dilute phenolic compounds. In regions with warm climates, varieties such as Cabernet Sauvignon and Feteasc㠠neagr㠠accumulate a high content of anthocyanins, which gives them an intense and stable color.

The results obtained in the main compound analysis indicate that PC1 explains 60% of the variation and PC2 explains 28%, which allows a clear separation of varieties according to anthocyanin and polyphenol content. Varieties with high anthocyanin values (Feteasc㠠Neagr㠠Cabernet Sauvignon) are located on the positive side of PC1, while varieties with high polyphenols (Olivia) are clearly differentiated on PC2 (Figure 1).

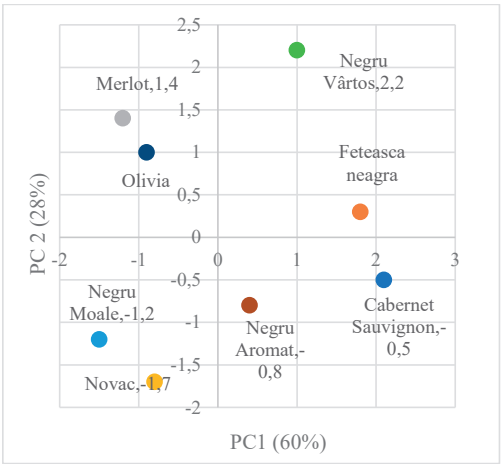


Figure 1. The distribution of the grape varieties according to PC1 and PC2

The highest anthocyanin content is found in the Novac (2326 mg/L) and Cabernet Sauvignon (1650 mg/L) varieties, which gives them a high potential for wines with an intense color and long stability.

PC1 separates varieties rich in polyphenols and anthocyanins (Novac, Cabernet Sauvignon) from those with a lower content (Merlot, Negru aromat).

PC2 reflects differences in tannin extractibility and phenolic maturity, indicating that Negru vartos and Merlot varieties have finer tannins and better extractability, while Novac and Negru moale exhibit more aggressive tannins and reduced extractability.

Feteasca neagra and Cabernet Sauvignon are positioned close to each other, suggesting similar phenolic characteristics and a good balance between structure and acidity.

Varieties on the negative side of PC2 (Novac, Negru aromat) tend to have a different phenolic profile, making them more suitable for distinct wine styles.

In order to understand the relationships between the analyzed variables, the analysis of the correlations between the phenolic characteristics of the grape varieties was performed.

The results show that there is a strong positive correlation between total anthocyanins (TA) and extractable anthocyanins (EA) ($r = 0.85$, $p < 0.01$), suggesting that varieties with a high content of total anthocyanins also have a high extractibility of them. Also, total polyphenols (TP) correlate positively with total anthocyanins and extractable anthocyanins ($r = 0.76$, $p < 0.05$), indicating that total polyphenols are influenced by these pigments. On the other hand, seed maturity (SM) has insignificant negative correlations with the other variables, which shows that seed maturity does not directly influence anthocyanin or polyphenol levels (Table 10).

The anthocyanin content is strongly influenced by temperature and rainfall, confirming the climatic impact on the stability of the pigments. Total polyphenols correlate significantly with sugars and acidity, suggesting a direct relationship between phenolic maturity and the chemical composition of grapes.

Extractability is largely determined by the chemical structure defined by PCA analysis, indicating a complex profile of phenolic interactions.

Table 10. The correlations between phenolic characteristics of the grape varieties in the period 2020-2024

Characte-ristic	TA	EA	EXTR*	TP	SM
AT	1.00	0.85***	0.42*	0.76**	-0.30
AE	0.85***	1.00	0.58**	0.62**	-0.25
EXTR	0.42*	0.58**	1.00	0.38	-0.15
PT	0.76**	0.62**	0.38	1.00	-0.20
MS	-0.30	-0.25	-0.15	-0.20	1.00

*EXTR-Extractibility
$p < 0.01$ (very significant correlation)
$p < 0.05$ (significant correlation)
$\hat{a}p < 0.1$ (marginally significant correlation)

CONCLUSIONS

Data analysis indicates some important trends that can guide future strategies in viticulture and winemaking:

The increase in average temperatures in recent years has favored the accumulation of anthocyanins, which indicates a possible intensification of the color of the wines produced.

Varieties with high acidity show greater stability over time, suggesting a high potential for aging.

Sugars and polyphenols are directly influenced by climatic conditions, which requires the adaptation of winemaking technologies to maintain aromatic and structural balance.

The regression between temperature and anthocyanins shows that in areas with warmer climates, varieties with a good adaptability can have a competitive advantage in the production of premium wines.

These trends suggest that climate change and variations in chemical composition need to be taken into account in cultivation and winemaking strategies to ensure consistent red wine quality.

Based on the observed trends and the relationships identified between the climatic variables and the chemical parameters of the grapes, a forecast can be made on the evolution of the oenological potential of the analyzed varieties:

Varieties with high sugar accumulation could become sweeter in the future, due to the

increase in average temperatures, which could require adjustments in winemaking technology to maintain the alcoholic balance.

Lower rainfall could lead to an increase in the concentration of polyphenols and anthocyanins, which would favor the production of more intense wines with a high aging capacity.

Varieties that are more sensitive to climate change may require technological adaptations, such as harvesting earlier or using cold maceration techniques to preserve phenolic compounds and acidity.

This forecast suggests that climate change will have a significant impact on the potential of red wines, and winemakers will need to adopt flexible strategies to maintain the quality and style of the wines produced.

Based on observed trends and climate change forecasts, the following recommendations can be made:

Adapting viticultural practices to optimize the accumulation of anthocyanins and polyphenols, given rising temperatures and variable rainfall.

Careful selection of varieties according to the wine region to maximize the potential of each type of wine produced.

Implementation of modern winemaking technologies, such as cold maceration and fermentation temperature control, to maintain the aromatic balance and stability of phenolic compounds.

Constant monitoring of climate change and adjustment of harvesting strategies to maintain optimal acidity and balanced sugar content.

In conclusion, this analysis provides essential information for winegrowers and oenologists, helping to develop superior quality wines, adapted to changing climatic conditions and international market requirements.

Comparative analysis of the chemical characteristics of red grape varieties revealed significant differences between them. These results can be useful for winemakers in selecting the right varieties according to the type of wine they want and for improving winemaking technology strategies.

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INVESTIGATION OF THE RELATIONSHIP BETWEEN SPECTRAL VEGETATION INDICES AND CHLOROPHYLL CONTENT IN THE LEAVES OF SYRAH CULTIVAR VINES

Anelia POPOVA, Zhulieta ARNAUDOVA, Zhivko GAVAZOV

Agricultural University, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: aneliyapopova@abv.bg

Abstract

Chlorophyll is a vital component of vine leaves, offering insights into their physiological state. It is the catalyst for photosynthesis, a process that drives the vegetative growth of vine plants. The present study investigates the correlation between chlorophyll content in vine leaves, which were simultaneously monitored by ground-based measurements and Sentinel 2 high-resolution vegetation indices (VIs)NDVI, CVI, CLGR through multispectral imaging. The experiment was conducted on Syrah grapevines over a three-year period, from 2020 to 2022. The vineyard was in full fruiting at the time of the study. In situ measurements of the photosynthetic activity of the vines were performed twice during the vegetative phase, before and after the flowering phase, in real conditions with a mobile apparatus (CCM-300). The remote data and imageries were processed using SNAP and QGIS. This study is of significant value to the field of precision viticulture, particularly in the context of grape production, as it provides insights into the potential of vegetation indices based on in situ measurements and VIs data from satellites.

Key words*: Chlorophyll, precision viticulture, remote sensing, vegetation index, vine.*

INTRODUCTION

Precision agriculture is dependent on several factors, one of which is remote sensing, which allows the health and condition of vegetation to be monitored. Many researchers in the field have analysed plant monitoring applications, research and scientific methodologies in this area (Giovos et al., 2021). The concept of precision viticulture is beginning to impact the wine sector, with several monitoring studies having been conducted in viticulture using a combination of vegetation indices, different remote sensing methods and in situ measurements (Arnó Satorra et al., 2009). The spatial variability of vineyard vigour has been assessed using the Normalized Difference Vegetation Index (NDVI) (Santesteban et al., 2013). Vegetation indices are primarily employed for the purpose of monitoring vine conditions, with the objective of correlating them with yield and other parameters such as chlorophyll concentration in leaves, nitrogen content, and water content in relation to canopy (Caruso et al., 2017; Gil-Pérez et al., 2010; Romero et al., 2018). Chlorophyll estimates derived from remote sensing are utilised in precision viticulture. It has been established that chlorophyll has a correlation with vegetation spectral indices estimated according to digital image analysis (Bodor-Pesti et al., 2023). However, the results of the correlation of pigments with chlorophyll concentration do not always coincide (Romero et al., 2018). The studies carried out with the chlorophyll concentration in the leaves of the vine variety 'Hárslevelű' (*Vitis vinifera)* examined the relationship of different vegetation indices. The authors of the study concluded that the deficiency of mobile elements is evident in older leaves, while immobile elements manifest symptoms in young organs such as the shoot tip (Bodor-Pesti et al., 2023).

Chlorophyll is the molecule in the chloroplasts of all green-pigmented parts of plants that act as a catalyst for photosynthesis. It absorbs the strong blue spectrum of light as well as red, and converts sunlight into chemical energy. As a result, chlorophyll content is reported to directly affect crop growth and yield. The ability to detect changes in chlorophyll content facilitates the distinction of physiological

variations in plants through phenotyping crops and monitoring crop characteristics (Jayne et al., 2018). Chlorophyll content is of particular significance in the context of reduced fertilizer application, improved yield estimation, and, most crucially, precision agriculture (Naqvi et al., 2021).

The necessity to improve the quality and profitability of agricultural production is driving farmers to introduce new technologies. This is a useful step in the evolution of agricultural management systems through the introduction of new technologies such as multispectral remote sensing cameras (Whelan & McBratney, 2012). The use of VIs in agriculture is beginning to evolve rapidly and is significantly expanding its range of applications (Boiarskii & Hasegawa, 2019).

The aim of this work is to investigate the relationship between vegetation indices, which refer to the content of chlorophyll in leaves, and in situ measurements in vines in different clones of the cultivar Syrah.

MATERIALS AND METHODS

Study area

The experiment was carried out on the training experimental field of Agricultural University in the town of Kuklen, Plovdiv district situated in Central-South Bulgaria (Figure 1).

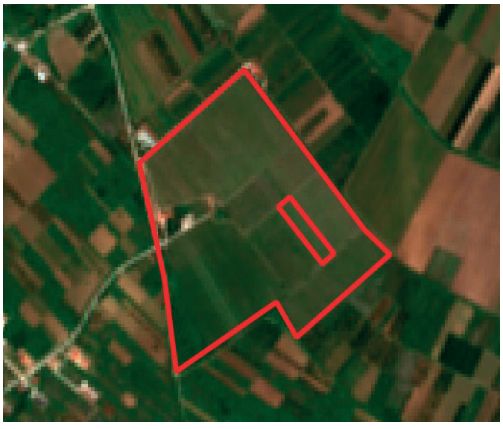


Figure 1. Location of study area
(Sentinel - 2 image 23.05.2021)
L 42° 3'29.71"N B 24°47'0.18"E

Experimental design and treatments

The images from Sentinel -2 were obtained from Copernicus browser and Vis were calculated by the formulas in Table 1. https://browser.dataspace.copernicus.eu/

Table 1. Formulas for Vis for chlorophyll contents in crops

Index name	Formula	Formula Sentinel-2	References
Chlorophyll Index Green (CLGR)	CLGR = (NIR/GREEN)-1	CLGR = (B8/B3)-1RED	(Gitelson et al., 2005)
Chlorophyll Vegetation Index CVI	CVI = (NIR/GREEN) *(RED/GREEN)	CVI = (B8/B3) * (B4/B3)	(Vincini et al., 2008)
Normalized difference vegetation index NDVI	NDVI = (NIR-RED)/ (NIR+RED)	NDVI = (B3-B4)/ (B8+B4)	(Rouse JW, 1974)

NIR - Near infrared light from electromagnetic spectrum

GREEN - Green light from electromagnetic spectrum

RED - Red light from electromagnetic spectrum

The vegetation indices are calculated from Sentinel-2 bands images and sample raster values were processed by SNAP and QGIS 3.10.

The correlations between the studied variables were obtained by regression analysis in Excel Microsoft 365 and were valid within the time range studied.

The present study utilised clones of the variety Syrah, namely numbers 100, 174, 470 and 524, which were grafted onto the rootstock Berlandieri x Riparia SO4. These clones were planted in April 2011 in the experimental training field of the Agricultural University in Plovdiv. *In situ* data collection was conducted between 2020 and 2022.

Experimental scheme includes the following 4 options:

V1 - Syrah variety, clone 174;

V2 - Syrah variety, clone 470;

V3 - Syrah variety, clone 524;

V4 - Syrah variety, clone 100.

Each variant includes 60 vines (4 repetitions x 15 vines).

The climate data for temperature and precipitation has been recorded by the climate station in the plantation during the period April - June.
The determination of total chlorophyll content in plants was conducted using a portable apparatus, designated as CCM-300. Measurements were performed during the active growth stage of vines under controlled conditions, on vine shoots of uniform size, load, exposure and illumination.
The BBCH scale was utilised to ascertain the phenology of the vines, with the decimal coding for determining the phenological stages of vine growth being identified as follows: the first digit indicates the main growth stage, and the second digit indicates the secondary growth stage, corresponding to a sequential number or percentage value.
The growth stages in the research period are based on DOY (Table 2).

Table 2. BBCH codes and growth stages

BBCH codes	Growth stages	DOY 2020	DOY 2021	DOY 2022
1:16	1: Leaf development 16 - sixth leaves are open	131	130	130
5:57	5: Flower display 57- inflorescences fully developed, flowers separated	146	143	140
7:71	7: Fruit development 71-budding, young fruit beginning to set, remnants of flowers falling off	156	168	158
7:75	7: Fruit development 75- Clusters of grains are pea-sized	181	180	180

RESULTS AND DISCUSSIONS

In situ data

Climate data

The pivotal elements of climate that exert an influence on the vital functions of the vine plant are air temperature and precipitation. It has been demonstrated that heat conditions have the capacity to influence the normal course of physiological and biochemical processes during the vegetation of the vines.

During the study period, daily mean temperatures and precipitation were recorded and presented in ten daily periods.
The average temperatures recorded during the study period, over the course of several years, did not exhibit significant variations. However, the total precipitation showed considerable differences (Figure2).
In a temperate climate, the soil is adequately supplied with water when the rainfall is 400-600 mm/m^2 and is distributed relatively evenly throughout the year.
The average annual precipitation in the vineyard in 2020 was 572.4 mm, with the highest recorded rainfall occurring in April at 106.8 mm. In 2021, the mean annual rainfall for the study area was approximately 200 mm higher than the previous year, at 755.4 mm. The mean annual precipitation in 2022 for the area was 572.4 mm, with June being the rainiest month (103.8 mm).
The total precipitation recorded during the 2020 growth stage BBCH 5:57 was 0.4 mm, while the post-flowering BBCH 7:71 precipitation was 17.4 mm.
A comparative analysis of the precipitation data from 2021 and 2022 with that from 2020 reveals that the respective amounts were 13.6 and 41.4 mm and 13.4 and 41.4 mm (Figure 3).

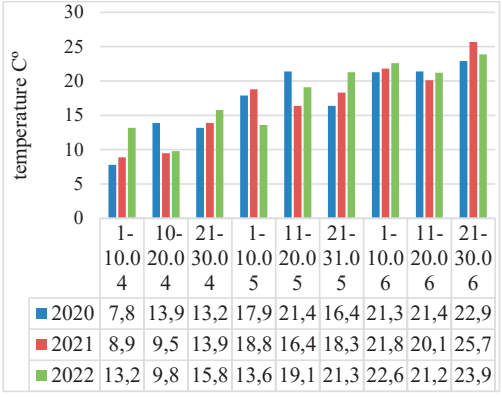


Figure 2. Average temperatures by ten-day periods from April to June

Chlorophyll measurements

The Syrah clones, numbers 100, 174, 470 and 524, were selected based on their identity, biological and production potential (ENTAV-INRA-ENSA-MONIVINS, 1995).

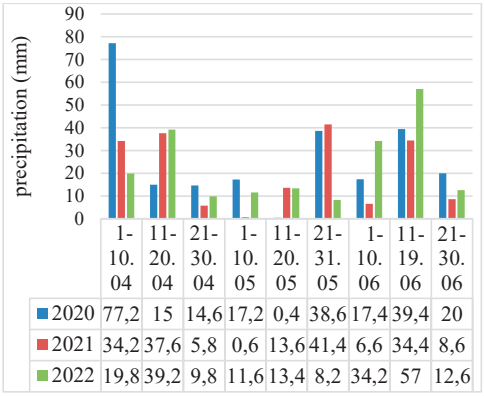


Figure 3. Precipitation totals in mm by ten-day periods from April to June

The content of plastid pigments (chlorophyll) in the tissues before the flowering stage of the vines is highest in the 0.470-0.366 g/m^2 clone, which also shows the highest net photosynthetic rate. The other three clones included in our study had lower chlorophyll content, with the lowest being at 100-0.316 g/m^2.

Following growth stage flowering, total chlorophyll levels remained relatively stable across the different vines, maintaining the same order as before flowering. Clone 470 exhibited the highest chlorophyll content of 0.271 g/m^2, which corresponded to a high transpiration intensity of 1.34 mmol H_2O m^{-2} s^{-1}. The other three clones displayed lower chlorophyll contents, with the lowest recorded in clone 100, at 0.246 g/m^2 (Table 3).

Vegetation indices relevant to chlorophyll

The chlorophyll leaf index CVI was proposed by Vincini et al. (2008) as a means of estimating the chlorophyll concentration in crop leaves.The leaf pigment of plants provides important information on their health status.

(Gitelson et al., 2005) developed a Chlorophyll Index Green CLGR to estimate the chlorophyll content in crops.

Plant reflectance values obtained by specialised methods are used to measure the content of, for example, chlorophyll, carotenoids and other nutrients (Gitelson et al., 2005). Chlorophyll, being capable of absorbing light energy, contributes to the photosynthetic process, whilst carotenoids protect photosynthetic systems from damage.

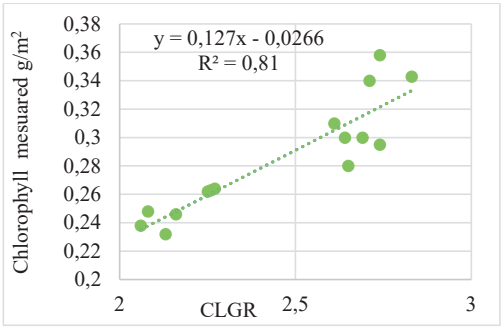
Table 3. Chlorophyll measured

BBCH CODE	V1 - Clone 174 Chlorophyll g/m^2	V2 - Clone 470 Chlorophyll g/m^2	V3 - Clone 524 Chlorophyll g/m^2	V4 - Clone 100 Chlorophyll g/m^2
2020				
1:16	0-247	0.264	0.262	0.263
5:57	0.343	0.358	0.340	0.295
7:71	0.238	0.246	0.248	0.232
7:75	0.310	0.300	0.280	0.300
2021				
1:16	0.250	0.268	0.270	0.270
5:57	0.326	0.335	0.335	0.287
7:71	0.248	0.248	0.235	0.233
7:75	0.300	0.330	0.320	0.300
2022				
1:16	0.313	0.298	0.282	0.270
5:57	0.356	0.405	0.374	0.367
7:71	0.263	0.321	0.273	0.274
7:75	0.284	0.290	0.276	0.343

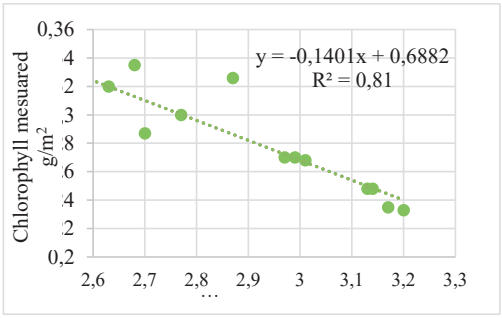
The Normalized Difference Vegetation Index (NDVI) is a widely used metric for assessing vegetation health and density. It measures the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs).

The study aimed to investigate the relationships between actual measured chlorophyll values and vegetation index CLGR, CVI and NDVI values obtained from Sentinel 2 imagery. The dates of the images obtained were as close as possible to the in-situ measurements.

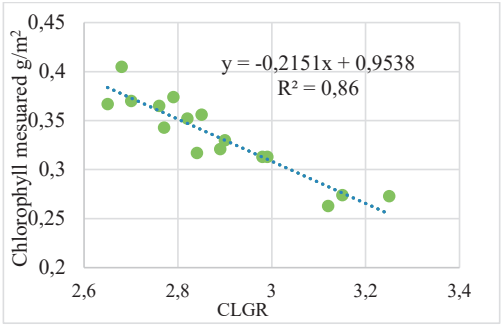
The regression analyses performed for the three-years study were processed between the CLGR, CVI and NDVI indices and the real in situ data of chlorophyll in the leaves of the vine cultivar clone Syrah are demonstrated in the figures given below (Figure 4, Figure 6, Figure 7).



a) 2020



b) 2021



c) 2022

Figure 4. Relationship between CLGR value from satellite and *in situ* data

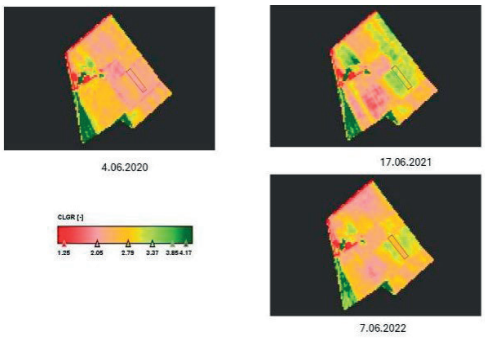
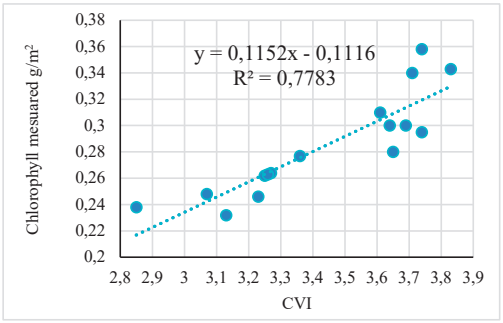


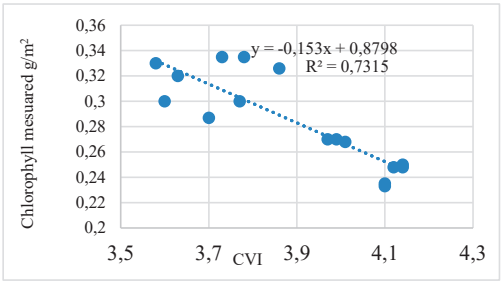
Figure 5. CLGR index from Sentinel 2 - BBCH 7:71 Fruit development

The range of CLGR index values across the experimental periods is from 2.1 to 3.3, with values in the first year of the period (2020) differing from those in the other two (2021, 2022). This is due to the higher temperatures and lack of or few amounts of precipitation in the period close to the chlorophyll measurements. The index ranges were from 2 to 2.9 in 2021 and from 2.6 to 3.3 in 2022. The multiple correlation coefficient is high for all three reporting years, $R^2 = 0.81$ and $R^2 = 0.86$.

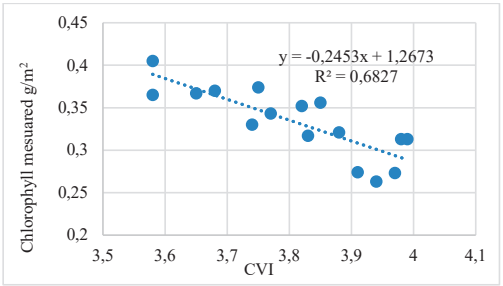
The scatter of values was observed in May during of the three years in growth stage BBCH 5:57 in clone 470 Syrah, and the best relationship was observed in phenological phase BBCH 7:71 (Fruit development) (Figure 5).



a) 2020



b) 2021



c) 2022

Figure 6. Relationship between CVI value from satellite and *in situ* data

The range of CVI index values for the three-year period under investigation is 2.8 to 4.2. The range of values for the chlorophyll vegetation index compared to the CLGR is largest in 2020.
In comparison, in 2021 and 2022 the index ranges from 3.5 to 4.2 and 3.5 to 4, respectively.
The strongest multiple correlation coefficient is reported in 2020, with a value of R^2 = 0.778. (Figure 6).
NDVIs are presented in a growth stage BBCH 7:71 Fruit development (Figure 8).

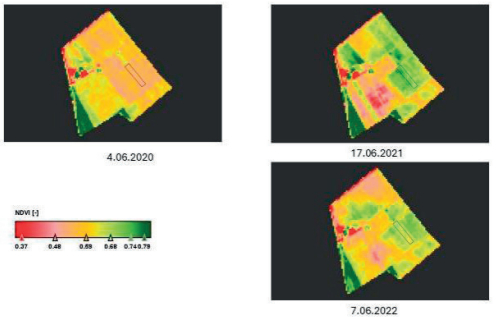
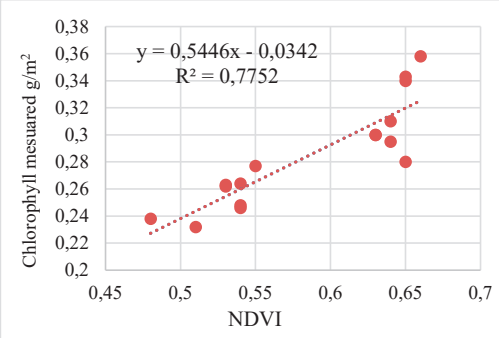
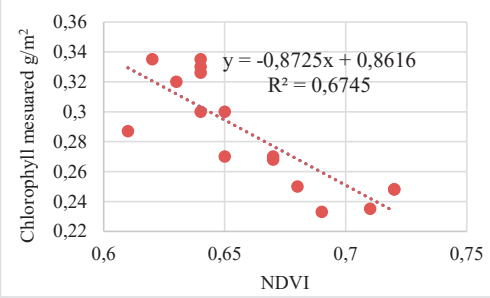


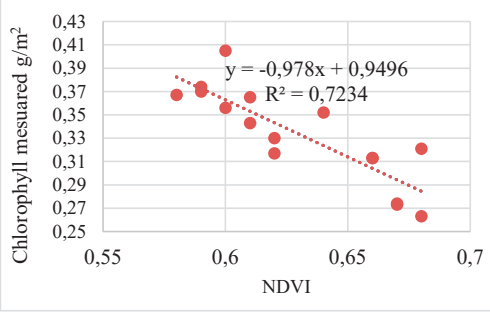
Figure 8. Figure 8. NDVI from Sentinel 2 - BBCH 7:71 Fruit development



a) 2020



b) 2021



c) 2022

Figure 7. Relationship between NDVI value from satellite and in situ data

The Normalised Difference Vegetation Index (NDVI) is the most common index for assessing plant vigour. Studies conducted during the experimental period demonstrated a very good multiple correlation coefficient of R^2 = 0.67 to R^2 = 0.77 for 2020. The widest range of values was reported for 2020, from 0.45 to 0.67, but in 2021, higher values of the index were reported (Figure 7).
This discrepancy can be attributed to the higher precipitation levels that occurred prior to the measurement of chlorophyll values and the acquisition of the image.

CONCLUSIONS

The application of the index comparison process resulted in the identification of a robust relationship and a high multiple correlation coefficient for CLGR, with values of R^2=0.81 and R^2=0.86, respectively.
The multiple correlation coefficient for NDVI is reported to be the lowest, with the smallest value recorded in 2020, R^2 = 0.67.
Despite the variations in the regression equations' values from those studied, the correlations proved to be of a very good degree.
The regression equations derived represent the relationships with *in situ* data and can be used to fit the values of satellite vegetation indices to analytical ones. This is extremely useful for estimating chlorophyll values with a high degree of probability and for making accurate predictions.
The primary advantage of CLGR, CVI and NDVI for practical applications is that they enable the use of RED, Green and NIR bands a

with a high spatial resolution (HR) of 10 m for the non-invasive monitoring of vine plantations, facilitating the assessment of physiological and biophysical parameters.

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STUDY ON THE IDENTITY OF A WINE BETWEEN VARIETY AND GEOGRAPHICAL SPACE

Marinela STROE, Diana Gabriela STAICU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: marinelastroe@yahoo.com

Abstract

The identity of a wine is the result of complex interactions between the grape variety and the geographical space in which it is grown. Understanding and capitalizing on these interactions are essential in the production of wines, which, beyond the exceptional quality, must reflect the specificity and authenticity of the place of origin, becoming an ambassador of the region and culture from which it comes. The sensory profile analysis in determining a wine's identity offers consumers an unparalleled multisensory and cultural experience by creating a unique and valued consumption experience. In this paper, the sensory characteristics that contribute to the identity of a wine were analyzed in detail, emphasizing aspects such as aromas, tastes, and the general structure of the wine. The results obtained provide a deep understanding of the organoleptic qualities of the wine and suggest distinct promotion strategies for each region, highlighting the specific strengths of each wine to satisfy varied consumer preferences.

Key words*: region, sensorial profile, terroir, variety, wine.*

INTRODUCTION

In the context of increased competition on the world wine market, against the background of globalization and the implicit consumer access to an increasingly rich offer of wine, but also of overproduction, the reference to the origin of the wine and, in particular, to the concept of terroir has become a factor differentiating and valuing the product. This aspect, as it improves the consumer's perception of the quality of the product and transfers to him the image and attitude regarding the region of origin (Aurier et al., 2005; Zindy et al., 2017). The prevalence of the notion of terroir as a marketing tool, particularly in the wine industry, indicates that this concept has an intrinsic commercial value, i.e. that it influences the consumer's purchase decision. In this sense, it was found that the perception of the authenticity of terroir products and the value associated with it by consumers have a positive and significant influence on consumer loyalty towards these products (Lacœuilhe et al., 2017). Although the definition of the concept of terroir is still a controversial academic topic, it is generally accepted that terroir products are perceived as authentic because they refer to a limited geographical area of production, within which the natural conditions and the know-how of the producer give special characteristics, defining the product. The preference for a certain product depends on the perception of the product's attributes, which in turn is significantly influenced by the attitude towards its region of origin (Laville, 1990; 1993; Zindy et al., 2017). However, despite the fact that the association of wine with terroir influences the decision to purchase it, it is difficult to appreciate to what extent consumers actually perceive the defining and specific attributes of a terroir wine to the extent that they do not know its origin in advance. Regardless of the definition given to terroir and despite its complexity, the concept of terroir is a valuable one, and the fact that controlled designations of origin, as a way of certifying quality, are relatively constant in terms of area covered, regulation and recognition, certifies the value of this concept. It was also found that terroir products are perceived by consumers as unique, having non-reproducible qualities: artisanal, recognizable, qualitative or different (Cadot et al., 2010; Casabianca et al., 2006; Spielmann & Gélinas-Chebat, 2012). As a consequence of this perception, consumers are willing to pay more to consume these products (Cross et al., 2011). Practically, in every wine-growing country,

where there are scientific arguments regarding the production of iconic wines, in different ecopedoclimatic conditions, it proves that each ecopedological factor involved contributes differently, leading to unique terroirs, different from each other (Barham, 2003; Gladstones et al., 2011; Stroe & Barcanu, 2011, Fabres et al., 2017; Van Leeuwen, C. & Seguin, 2006). In other words, in these areas there is a set of factors that interact and intervene, in a particular way, individualizing the terroir and giving the wine its originality. The development of this concept, the interaction between variety and terroir is one of the factors that, under the conditions of increased global competition, can lead to the increase in the production of original and authentic wines, bearing the signature of the place (Riou C. et al., 1995). The fact that one and the same grape variety can give rise to very different wines depending on where it is grown is a challenge, but also an opportunity for winegrowers and winemakers to experiment and optimize combinations of varieties and terroirs to obtain special and authentic wines. Descriptive sensory analysis is one of the most powerful, sophisticated and extensively used tools in sensory science, which provides a complete description of the sensory characteristics of food products that affect and direct consumer preferences (Varela & Ares, 2012). In general, professional evaluators are used, but considering the economic and time implications of training evaluators for descriptive analysis, they can be used with semi-trained evaluators or even consumers using the organoleptic description of red wines based on intensity scales, being able to appreciate the general and specific composition, color structure and indices of phenolic typicality, allowing a detailed and precise evaluation of each aspect of the wine. Sensory profile analysis in determining a wine's identity provides consumers with an unparalleled multisensory and cultural experience by creating a unique and valuable consumption experience. In this paper, the sensory characteristics of two wines obtained from the 'Merlot' variety, with different identities, were analyzed in detail, highlighting the general structure of the wine, as well as aspects related to their olfactory and gustatory notes.

MATERIALS AND METHODS

The wine world is full of grape varieties, each with its own unique characteristics, but in this work we have studied one of the most famous and appreciated varieties for red wines - 'Merlot'. This variety has spread throughout the world, and has become iconic due to its productive qualities and outstanding attributes highlighted by balanced, velvety and full-flavored wines. The variety is native to France (Bordeaux region), with origins in the 18th century, when it was first mentioned in historical documents (Burdea R.O. et al., 2020; https://www.vivc.de/index.php?r=passport%2Fview&id=7657. What makes it interesting for the wine-growing landscape in Romania is the high degree of adaptation in various regions, being cultivated in many wine-growing centers (76) practically in all those wine-growing areas favorable to the cultivation of varieties for red wines, in the south of Moldova, in the Region of the Muntenia and Oltenia Hills, in Dobrogea and insular in the eastern part of the country. In all these areas, quality wines are obtained that reflect the characteristics of the local terroir, being popular both as a single varietal and in blends, where it brings roundness and harmony. In Romania, it is grown on 11,108.71ha and the following synonyms are allowed for labeling: 'Plant Medoc' and 'Bigney rouge', according to the OIV 2013 list (number 2115) https://winesofromania.com/merlot-un-povestitor-de-cursa-lunga/. The aromas and taste profile of 'Merlot' wine can vary depending on the terroir, the production methods and the maturation process, and the predominant aromas include (Figure 1):

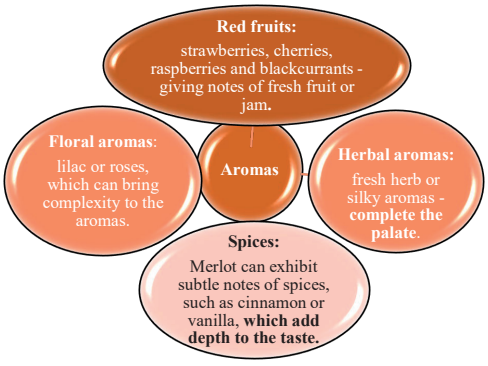


Figure 1. Specific aroma 'Merlot' variety

In this paper, the analysis focuses on the testing of two distinct 'Merlot' wines from the 2018 harvest: one from Bordeaux, France (Château Marsau), and the other from the Drăgășani vineyard - Romania (Stirbey 'Merlot' Reserva Ordinul de Malta). Both wines were subjected to rigorous analyzes to determine their chemical parameters and, implicitly, the sensory profiles that define their identity.

Table 1. Experimental Variant (VN_1)



Country of Origin	France
Area	Bordeaux
Vintage	2018
Sugar	Dry
Color	Red
Serving temp.	16-18°C
Volume	0.75 L
Alcohol	14.8%
Grape variety	Merlot
Color	Ruby red
Nose	Cherries, wild berries, black olives, lightly roasted coffee beans
Taste	Bouquet of wild berries, cinnamon, and coffee beans
Food paring	Cheeses, mutton, pork, game, beef
Price:	43.55 EUR (supermarket)

The tasting was attended by 10 tasters, of which 5 hold internationally recognized certifications such as WSET (Wine & Spirit Education Trust) Level 3 or higher, and have attended advanced oenology and sensory analysis courses, and these qualifications demonstrate in-depth knowledge of wine chemistry, the winemaking process and tasting techniques, and 5 consumers.

Before the tasting session, the tasters were instructed in detail about the descriptive tasting sheet used for the red wines, the methodology and the objectives of the study.

Participation was voluntary and anonymous, and no personal data that could be linked to respondents was collected.

The analyzes were carried out at a private laboratory in Urlati, county Prahova and the that were methods used are presented in Table 3. The terroir elements (other than the cultivated variety) specific to the two areas subject to the study (Bordeaux and Dragasani) are presented synthetically (Table 4) in order to be able to later, when the results of the study reveal a distinction in the sensory profile for the two wines, draw certain conclusions regarding their contribution to the degree of distinction.

To analyze the distinctiveness of the wines under study, the One-way ANOVA analysis was used to evaluate the significant differences between the various aromatic, olfactory and gustatory characteristics.

Table 2. Experimental variant (VN_2)



Country of Origin	Romania
Area	Dragasani
Vintage	2018
Sugar	Dry
Color	Red
Serving temp.	15-18°C
Volume	0.75 L
Alcohol	14.7%
Grape variety	Merlot
Color	Ruby red
Nose	Fine tannins, aromas of black pepper and dark chocolate
Taste	Bouquet of ripe blackberries and plums, cinnamon and coffee beans
Food paring	Cheeses, mutton, pork, game, beef
Price:	25.33 EUR (supermarket)

Table 3. Parameters analyzed and methods used

Parameters	Methods used
Alcohol concentration	Standard 6182/6-70
Relative density at 20°C	Standard 6182/8-71
Total dry extract	Standard 6182/9-80
Total acidity	Standard 6182-1:2008
pH	SR 6182-14:2009
Volatile acidity	SR 6182-2:2008
Sucrose+D-Glucose+D-Fructose	SR 6182-18:2009 Enzymatic determination
Free sulphur dioxide (free SO_2)	SR 6182-13:2009
Total sulphur dioxide	SR 6182-13:2009
Calcium	Colorimetric determination
Copper	Colorimetric determination
Potassium	Turbidimetric determination
Iron	Enzymatic determination
Glycerol	Colorimetric determination

Table 4. Terroir elements specific to the studied areas

	Bordeaux	Drăgășani
The delimited geographical space	The Bordeaux region is located in the southwest of France. Along the Gironde estuary, being crossed by the Garonne and Dordogne rivers, located on the parallel of 45 degrees (44.84 north latitude). 120,000 hectares of vineyards, 32% of the cultivated area is cultivated with the Merlot variety.	The Drăgășani vineyard is located in the region between the Getic Subcarpathians to the north and the Romanian Plain to the south and southeast, which stretches for a length of approximately 50 km, and a width of up to 30 km in the east-west direction, it is located between parallels 44°30´ - 44°55´ northern latitude.
Relief	The relief is varied, with hills and valleys providing different sun exposures and microclimates, thus influencing the styles of wine produced in different parts of the region.	Located on hills and hills, the region benefits from varying sun exposures and diverse microclimates, similar to those of Bordeaux, contributing to the variety and complexity of the wines produced.
Soil	Soils vary, including clay, limestone and gravel. These soils allow good drainage, essential for the vines, contributing to the development of deep roots and a wine with a distinct character.	The soils are diverse, but clayey and calcareous predominate. These soils are favorable for growing vines, ensuring adequate drainage and providing important mineral elements that contribute to the complexity of the wines.
Climate	It has a moderate oceanic climate, influenced by its proximity to the Atlantic. Temperatures are relatively mild, with hot summers and mild winters, and rainfall is evenly distributed throughout the year.	It has a temperate-continental climate with Mediterranean influences. Summers are hot and dry and winters are mild, similar to Bordeaux in moderating thermal extremes.
Qualities and peculiarities imprinted on the wines, typicality and authenticity	'Merlot' from Bordeaux, especially from areas such as Saint-Émilion and Pomerol, benefits from clay soils and a moderate climate, producing round wines with black fruit flavors and velvety tannins.	Although Drăgășani is not as well-known as Bordeaux, the 'Merlot' variety grown here can offer wines with similar characteristics, benefiting from the clay-limestone soils and temperate-continental climate. The wines can have aromas of red and black fruits, with a balanced structure and soft tannins.
The similarities between the terroir of Bordeaux and that of Drăgășani are manifested in the moderate climate, diverse soils and varied topography, all contributing to the production of quality wines. Although there are regional and specific differences in each terroir, these similarities allow the cultivation of similar grape varieties, such as 'Merlot', and obtaining wines with comparable characteristics in terms of complexity and balance.		

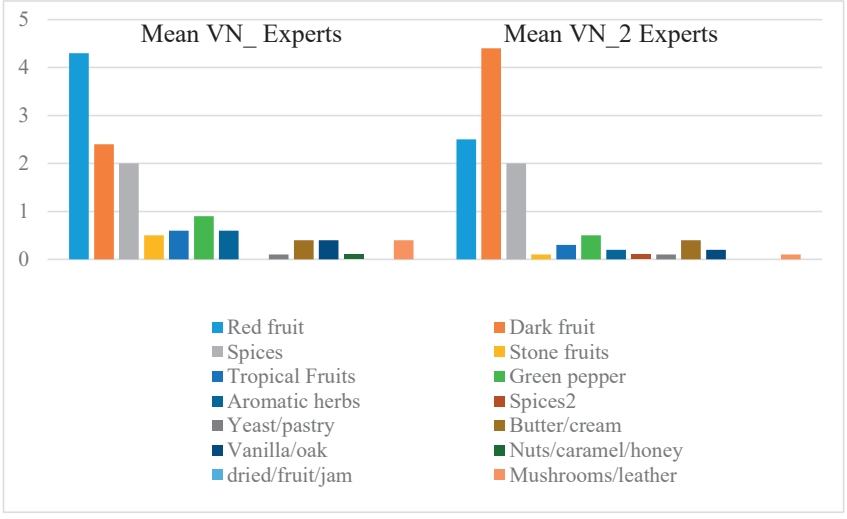


Figure 2. Average of the main olfactory characteristics according to experts

RESULTS AND DISCUSSIONS

The main element that is consistently found in the definitions given to terroir is the delimited geographical area associated with it, but the cultivated grape variety is part of the biological factors that configure the terroir (Table 5). According to the specialized literature (Roland S., 2019; van Leeuwen et al., 2020, van Leeuwen et al., 2022) the chemical composition of 'Merlot' wine can vary depending on the harvest years and the winemaking techniques used. Thus, we can provide the general values for the main chemical parameters characterizing the two wines (Table 5).

Table 5. Chemical parameters of the analyzed wines

Parameters	VN_1 Bordeaux	VN_2 Dragasani	Specification
Alcohol concentration	14.8	14.7	% vol.
Relative density at 20°C	0.9927	0.9929	g/ml
Total dry extract	29.0	29.7	g/l
Total acidity (acid tartaric)	4.91	5.24	g/l
pH	3.61	3.42	-
Volatile acidity (acetic acid)	0.53	0.5	g/l
Residual sugar	2.35	1.31	g/l
Free sulphur dioxide (free SO_2)	5.46	6.21	mg/l
Total sulphur dioxide (SO_2)	43.77	47.59	mg/l
Calcium	64.4	72.1	mg/l
Copper	0.015	0	mg/l
Potassium	1206	816	mg/l
Iron	3.79	0.91	mg/l
Glycerol	6.81	7.63	g/l

Wine analysis VN_1. The wine from Bordeaux, 'Merlot' 2018 has an alcohol concentration of 14.80% vol, an indicator of the maturity and intensity of the fruit. Relative density at 20°C is 0.9927, which suggests a well-balanced and structured wine. Total dry extract of 29.0 g/l indicates a wealth of compounds that contribute to the complexity and body of the wine. Total acidity (tartaric acid), measured at 4.91 g/l tartaric acid, is another essential parameter that influences the freshness and longevity of the wine. With a pH of 3.61, this 'Merlot' maintains sufficient acidity to ensure a pleasant taste balance, while the Volatile acidity (acetic acid) of 0.53 g/l acetic acid suggests a well-controlled fermentation and a clean winemaking process. Residual sugar (Sucrose+D-Glucose+D-Fructose) of 2.35 g/l adds a subtle note of sweetness, balanced by acidity, contributing to a harmonious taste profile. Free sulphur dioxide (free SO_2) and Total sulphur dioxide (SO_2) is also an important indicator of wine stability and shelf life. The values of 5.46 mg/l for free sulphur dioxide and 43.77 mg/l for total sulphur dioxide are within food safety limits, ensuring adequate protection against oxidation and microbial growth. The content of calcium (64.4 mg/l) and copper (0.015 mg/l) are within normal limits, contributing to the stability of the wine, while potassium (1206 mg/l) and iron (3.79 mg/l) play essential roles in biochemical and sensory processes. Glycerol, with a value of 6.18 g/l, adds body and a feeling of softness to the taste, thus amplifying the positive sensory perception. All these chemical components combine to create a complex wine with intense fruity aromas, soft tannins and a balanced structure, reflecting the unique terroir of Bordeaux.

Wine analysis VN_2. On the other hand, the 'Merlot' wine from Drăgășani presents distinct characteristics, influenced by the Romanian terroir (Stroe & Dîmboviceanu, 2020). With an alcohol concentration of 14.70% vol and a relative density at 20°C of 0.9929, this wine shows a similar balance to its french counterpart, but with different subtle nuances. Total dry extract of 29.7 g/l suggests a slight superiority in terms of the richness of solid compounds, which may influence the texture and complexity of the taste. Total acidity (tartaric acid) of 5.24 g/l tartaric acid and pH of 3.42 indicate a more acidic and fresher profile, which can contribute to a more vibrant feel and increased longevity of the wine. Volatile acidity (acetic acid) of 0.50 g/l acetic acid is slightly lower, reflecting a well-managed fermentation. Residual sugar (Sucrose+D-Glucose+D-Fructose) of 1.31 g/l is lower compared to Bordeaux wine, which can influence the perception of sweetness and taste balance. Free sulphur dioxide (free SO_2) and total sulphur dioxide are 6.21 mg/l and 47.59 mg/l, respectively, slightly higher than the values corresponding to French wine, providing additional protection against oxidation. The levels of calcium (72.1 mg/l) and copper

(0 mg/l) show significant differences in the mineral composition, while potassium (816 mg/l) and iron (0.91 mg/l) reflect the natural variations of the terroir and the winemaking techniques used. Glycerol, with a value of 7.63 g/l, is significantly higher, contributing to the sweetness and body of the wine. These chemical and sensory differences create a wine with distinct aromas, which reflect the influence of the terroir and the craftsmanship of the Drăgășani winemakers. In general, the 'Merlot' wine profile can include notes of red and black fruits, with well-integrated tannins and a fresh acid structure. Regarding the evaluation of the impact of chemical parameters on the sensory profile, the comparative analysis of these two wines highlights how the terroir, viticultural traditions and winemaking techniques can influence the final characteristics of the wine. Bordeaux wine, with an emphasis on aromatic complexity and taste balance, reflects a well-established tradition of producing superior quality wines. The wine from Drăgășani, with its acidic profile and velvety texture, shows the potential of the Romanian region to produce distinctive and high quality wines. These observations underscore the importance of the sensory profile in defining a wine's identity, providing both producers and consumers with a deeper understanding of the qualities that make a wine unique. The rigorous analysis of these chemical parameters and their correlation with the sensory characteristics, from this study contributes to the identification and comparison of the sensory characteristics. The identity of a wine between variety and geographical space is highlighted in the sensory profiles of the two wines from the perspective of the main olfactory characteristics according to the experts (figure 2), the VN_1 variant shows a greater intensity of red fruits compared to VN_2 indicating a more pronounced presence of these notes in its olfactory profile. Both wines have high values for red and black fruit aromas, but the Bordeaux wine is rated as having a slightly higher intensity, identifying empyreumatic notes. Spices aromas, such as blackpepper and dark chocolate, are more evident in the Dragasani Wine. These aromas are less perceived, suggesting that the maturation processes and oak influences are less dominant in the profile of this wine.

Profiles configured by experts provide a detailed insight into the dominant notes and contribute to a better appreciation of the sensory quality of wines. Analyzing the average of the main olfactory characteristics according to consumers (Figure 3) VN_2 presents a greater intensity of dark fruit aromas compared to Bordeaux wine (VN_1) indicating a more pronounced presence of these notes in its olfactory profile. Basically, both wines have high values for dark fruits and red fruits aromas, VN_2 having a slightly higher intensity, and VN_1 having a higher intensity of spices aromas. Regarding the perception of the taste characteristics - from the point of view of the experts (Figure 4) VN_2 presents a greater intensity of dark fruits (blackberries and plums), aromas compared to VN_1. Both wines have quite high values for the aromas of dark fruits and red (cherries, raspberry) VN_1 also having a higher intensity for the aromas of aromatic spices. Green pepper and aromatic herbs are present in both wines, but with reduced intensities. The other flavors of spices, yeast/pastry, butter/cream have low intensities in both wines, reflecting an aromatic profile more focused on fruit and citrus notes. Vanilla/oak, nuts/caramel/ honey, dried fruits/jam, mushrooms/leather are barely perceived, suggesting that maturation processes and woody influences are less dominant in the profiles of these wines. From the perspective of consumers (Figure 5) VN_1 and VN_2 present intensities quite close to dark fruits and red fruits, with VN_1 being slightly superior. The VN_2 wine has a greater intensity of aromas of black fruits and spices (cinnamon and coffee beans) compared to VN_1 where the aromas of red fruits predominate.

Distinctiveness check. From this point of view - one-way ANOVA analysis was used to evaluate the significant differences between the different olfactory aromatic characteristics. The results show that the highest intensity is also for dark fruit and red fruits, and herbal aromas are present in both wines, but with low and very low intensities.

Spices, yeast/pastry and butter/cream have low intensities in both wines, reflecting an aromatic profile more focused on fruity notes. Vanilla/oak, nuts/caramel/honey, dried fruits/gem and mushrooms/leather are less perceived or not at all, which suggests that the maturation processes and oak influences are less

dominant in the profiles of these wines. The average values and standard deviations show that both experts and consumers they perceived aromas of red fruits and dark fruits in both wines, with a slightly stronger intensity mare pentru VN_2 (Table 6). Experts reported a higher intensity of dark fruit flavors in VN_1 - while consumers perceived similar intensities for both wines.

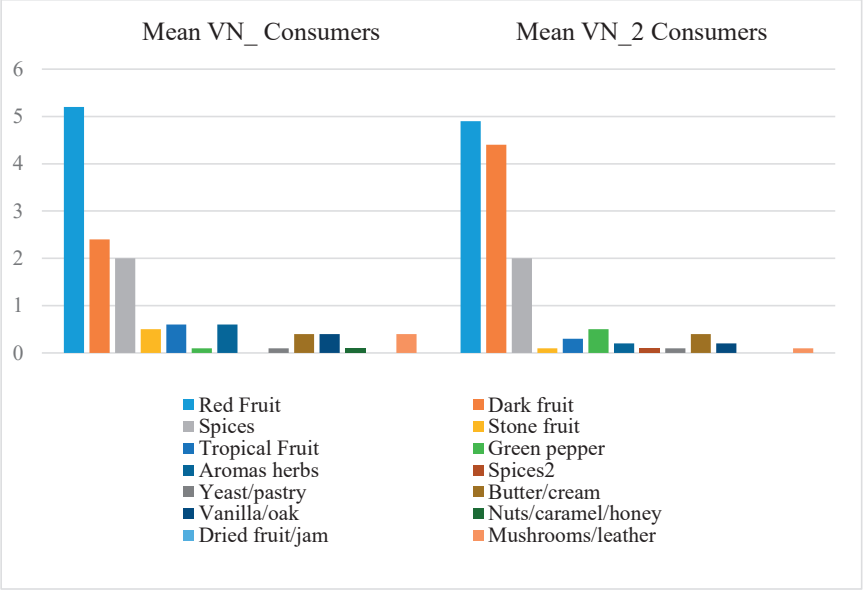


Figure 3. Average of the main olfactory characteristics according to consumers

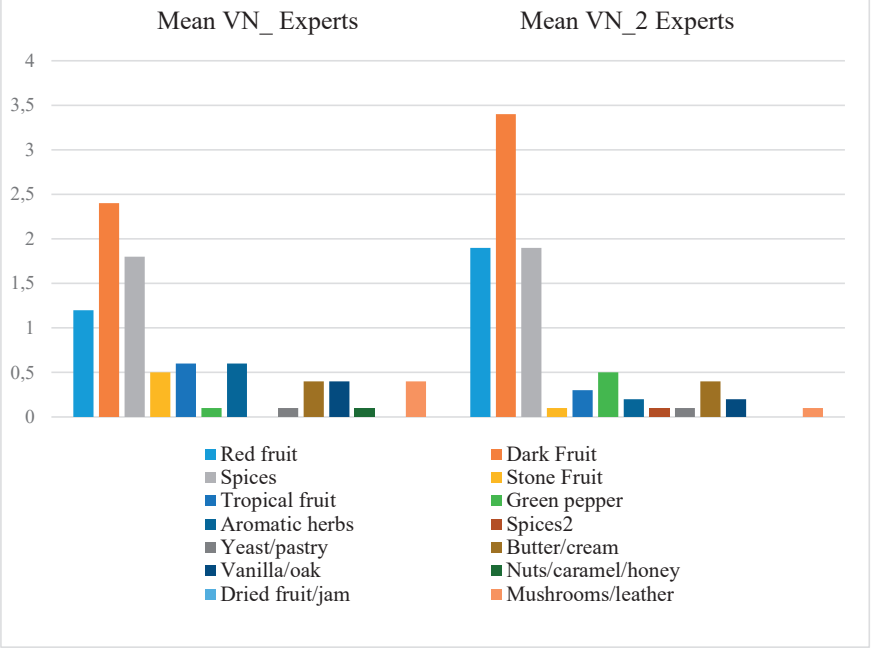


Figure 4. Average of the main taste characteristics according to experts

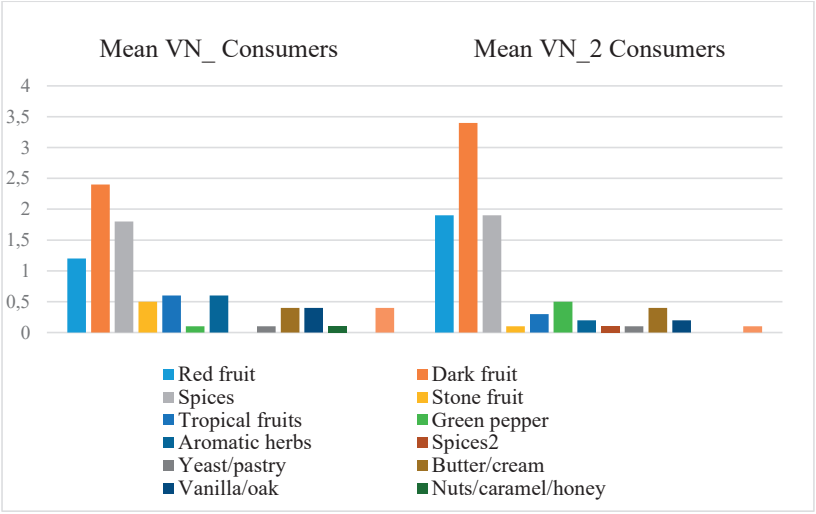


Figure 5. Average of the main taste characteristics according to consumers

Table 6. Synthesis of the aromatic olfactory characteristics of wines VN_1 and VN_2 (ANOVA test)

	Experts		Consumars	
Descriptor	VN_1	VN_2	VN_1	VN_2
Dark fruit	2.5 ± 0.5	2.6 ± 0.4	1.7 ± 0.3	2.0 ± 0.3
Red fruit	2.4 ± 0.3	1.8 ± 0.2	1.8 ± 0.4	1.9 ± 0.4
Spices	2.9 ± 0.4	2.5 ± 0.3	2.4 ± 0.3	2.5 ± 0.3
Stone fruits	1.9 ± 0.5	1.5 ± 0.4	1.0 ± 0.4	1.1 ± 0.3
Tropical fruits	1.0 ± 0.3	1.1 ± 0.3	0.6 ± 0.2	0.7 ± 0.2
Green pepper	0.7 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
Aromatic Herbs	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.4 ± 0.1
Citruses	0.0 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Yeast/pastry	0.3 ± 0.1	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Butter/cream	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Vanilla/oak	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
Nuts/caramel/ honey	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Dried fruit/jam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Mushrooms/ leather	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Table 7. Summary of consumer preferences for wines VN_1 and VN_2

Descriptor	VN_1	VN_2
Dark fruit	1.7 ± 0.3	2.0 ± 0.3
Red fruit	1.8 ± 0.4	1.9 ± 0.4
Spices	2.4 ± 0.3	2.5 ± 0.3
Stone fruits	1.0 ± 0.4	1.1 ± 0.3
Tropical fruits	0.6 ± 0.2	0.7 ± 0.2
Green pepper	0.3 ± 0.1	0.3 ± 0.1
Aromatic Herbs	0.0 ± 0.0	0.4 ± 0.1
Citruses	0.3 ± 0.1	0.4 ± 0.1
Yeast/pastry	0.0 ± 0.0	0.0 ± 0.0
Butter/cream	0.2 ± 0.1	0.3 ± 0.1
Vanilla/oak	0.1 ± 0.0	0.2 ± 0.1
Nuts/caramel/honey	0.1 ± 0.0	0.0 ± 0.0
Dried fruit/jam	0.0 ± 0.0	0.1 ± 0.0
Mushrooms/leather	0.2 ± 0.1	0.2 ± 0.1

Both groups reported values for spice aromas, with VN_1 having a slightly higher intensity. Both experts and consumers perceived low intensities of stone fruit aromas, with a slight favorable difference for VN_1. Tropical fruits are more evident in VN_1, although the differences are not very big between the two wines. Both groups reported reduced intensities for Gren pepper, slightly reduced however for VN_2. Experts reported higher values for VN_2 compared to VN_1, while consumers perceived low and similar intensities.

Flavors of spices (lightly roasted coffee beans) are more evident in VN_2 for both groups.

Experts reported low intensities for both wines, with a slight preference for VN_2. As for the vanilla/oak aroma, they were perceived at low intensities by both groups. Experts reported low intensities for both wines, while consumers perceived these aromas as very low. For nuts/caramel/honey experts reported higher values for VN_1, imperceptible in VN_2, while consumers did not perceive these aromas in either wine. To identify consumer preferences among the wines under study (Table 7) it emerged that consumers slightly prefer the black fruit aromas of wine VN_2 - compared to VN_1 - suggesting that a more pronounced fruity

aromatic profile is appreciated. Both wines have similar intensities of black and red fruit aromas but VN_2 has a slight preference. Aromas of spices are appreciated in both wines, with a slightly higher preference for VN_2.
Consumers do not show a significant preference for stone fruit flavors in any of the wines, but VN_2 is slightly preferred. Tropical fruits flavors are appreciated at low intensities, with VN_2 having a slight preference. Consumers prefer the VN_2 wine for these aromas, although the intensities are reduced. Consumers slightly prefer the Vanilla/oak aromas in VN_2, although even the VN_1 wine has a slight intensity. Preference for nuts/caramel/honey, dried fruits/jam, mushrooms/leather is very low, with a slight preference for VN_1.
Appreciated globally, based on the results in table 7, the preferences from this point of view are similar for both wines. Analysis of consumer preferences shows that VN_2 wine is slightly preferred for most aroma descriptors, especially red and black fruit, spice and butter/cream aromas.
These preferences can help winemakers better understand which flavors are more appreciated by consumers and adjust production processes accordingly to improve customer satisfaction.

CONCLUSIONS

The identity of a wine is the result of a complex synergy between the grape variety and the geographical space in which it is grown and the wine reflects the specificity and authenticity of the place of origin.
The concordance between Experts and Consumers suggests that perceptions of wines are consistent regardless of tasters' level of knowledge.
Bordeaux Region through 'Merlot' Wine, 2018 has an identity linked to complexity, refinement and maturation potential and is preferred for special occasions, taking into account the purchase price, appreciated by consumers looking for depth and structure.
The Drăgășani Region through 'Merlot' Wine, 2018 has an identity related to freshness, accessibility and immediate pleasure, preferred for daily consumption and informal moments (with the affordable price), being appreciated by consumers looking for a pleasant, balanced wine.
Considering the price difference, the increasingly educated consumer will choose consciously, depending on the context, wanting to find the desired quality in the price.
Even if the decision-making factors in the choice of wine are basically based to a greater extent on elements external to the wine - label, brand, price, the decision in choosing a wine is rather one based on the previous experience with the wine or the recommendations of friends, which shows that consuming wine seems to be more of a social habit, which is educated over time.
In the consumer's purchase decision, beyond the price, driven or not by a well-structured marketing, there is also the concept that if you know where the wine comes from wine, what variety, how it was produced, what kind of technology was used, you will appreciate it, and finally, you will buy it.
Each bottle of wine becomes an ambassador of the region and culture from which it comes, offering consumers an unparalleled multisensory and cultural experience.
The results obtained provide a deep understanding of the organoleptic qualities of the wine, for each region, highlighting the specific strengths of each wine to satisfy the rather varied preferences of consumers.

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STUDY ON THE BEHAVIOR AND DEGREE OF ADAPTABILITY OF THE MATILDE VARIETY IN THE CLIMATE CONDITIONS IN ROMANIA

Marinela Vicuța STROE[1], Georgeta Mihaela BUCUR[1], Steliana Paula BARBU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research and Developement Institute for Viticulture and Enology Valea Călugărească,
2 Valea Mantei Street, Valea Călugărească, Prahova, Romania

Corresponding author email: marinelastroe@yahoo.com

Abstract

The Romanian wine-growing area, through all the terroir conditions offered, ensures the cultivation of a varied range of table grape varieties, both from the international and domestic assortment, thanks to the generosity of the orographic, climatic, pedological factors and, last but not least, the applied technology, rich in innovative measures to ensure a good plant-productive balance. In the present work, the observations, measurements and analyzes focused on the evaluation of the agrobiological and technological parameters of the 'Matilde' variety, verified by calculating specific and representative indices that provide clues about the adaptation of this variety to the climatic conditions of southeastern Romania, different from of the place of origin of the variety. The results obtained, supported by the attributes and quality of the production, offer a positive perspective, which can include the variety in the planting list of economic agents who grow table grapes.

Key words*: adaptation, climate change, grape, quality, yield.*

INTRODUCTION

Of a world production of 80.1 Mt, 45.7% is destined for wine production, 47.4% for direct consumption as table grapes and only 6.9% for dried grapes, and the rest for the production of musts and juices (OIV, 2024). The increasingly high demands and preferences of consumers for table grapes are generally directed towards quality attributes, seen from a compositional perspective (Crespo et al., 2024; Seccia et al., 2019; Di Lorenzo et al., 2019), but also towards those related to aesthetics - the shape, size and shape of the berries, the shape, size and bunch density, uniformity of size, the firmness of flesh, taste and particular flavor (strong Muscat aromas), the uniformity of color, at full maturity (Rolle et al., 2011; 2013), the ripening period or any other qualitative attribute specific to grapes (Cichi et al., 2023; Benbouguerra et al., 2021; Dobrei et al., 2020; Tahereh et al., 2020). Therefore, producers must align their offers with consumer expectations and increase competitiveness and market dynamics. In order to meet this objective, the curators of ampelographic collections, upon the immediate appearance of news created varieties, take them into agronomic observation so that they can be evaluated from a quantitative and qualitative point of view. Most of the time, institutions holding viticultural germplasm are located in areas fundamentally different from the areas where the varieties were obtained, and then it is imperative to monitor the size of production in order to better quantify the notion of quality and competitive product. Therefore, ampelographic collections have the essential role of preserving and saving vine planting material and maintaining varietal biodiversity, and they also have a role in testing these varieties. The scientific results obtained here will be disseminated and transferred to economic agents who cultivate table grapes and will play a significant role in the decision-making process to enrich the varietal assortment of each country, shaping the dynamics of the consumer market (Cichi et al., 2019; Seccia et al., 2019). The study was approached starting from two considerations: determining and evaluating the agrobiological performances of the 'Matilde' variety (obtained in Italy) cultivated in an area different from its

origin, in the specific conditions of the South-East of Romania, and quantifying the productive balance in terms of defining quality parameters (Pârcălabu, 2010, cited by Stroe & Budescu, 2013). Basically, the idea was that, regardless of the origin of the varieties, their vigor, production capacity - their adaptability is also given by their genetics and how they are influenced by climatic and agrotechnical factors. Therefore, in studies that follow the behavior of grapevine varieties in areas different from their place of origin, more and more attention is paid to research that targets the mode of vegetative-productive manifestation, as a direct result of physiological processes and applied cultural practices.

MATERIALS AND METHODS

Plant Material and Growth Condition

The research was carried out in two years wine growers 2022-2023 and 2023-2024 at the located in the experimental field of the ampelography collection from University of Agronomic Sciences and Veterinary Medicine of Bucharest, with the code "ROM 06" in international platform the https://www.vivc.de/ (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m). The variety studied was the ‚Matilde' recognized for its precocity, productivity and special organoleptic qualities, which in our country we find only in ampelographic collections.https://www.vivairauscedo.com/en/product-sheet/matilde/. It was obtained in 1962 by Manzo P. at the Horticultural Research Institute of Roma. The genitors are ‚Italia' and ‚Cardinal'.The variety has a medium-large, ovoid and the color of skin is white. The flesh is crispy flesh with a discreet muscat flavor, and is used in its country of origin as a table variety, consumed fresh and in compotes. https://www.vivc.de/index.php?r=passport%2Fview&id=7512. The plantation planted in 2018 at a distance by 2.2 m (inter-row) and 1.2 m (intra-row), with a density of 3787 plant ha-1. The type of pruning applied is double Guyot on the stem 1.0 m, and the load distributed on the plant was 32 buds/vine. The variety was grafted on SO4-5 rootstock. During the vegetation period, a whole set of green special and operations specific to the table grape ensuring adequate management adapted to the growth vigor of this variety. The crop was managed with irrigation in critical phenophases (intense berry growth), fertilizer application and a standard disease control program was applied to control downy mildew (*Plasmopara viticola*), and gray rot (*Botrytis)*.

Sampling and Chemical Analysis

For the proposed objective - monitoring the behavior of this variety in the conditions of southeastern Romania, observations and determinations were made regarding the calendar course of the phenological spectrum and observations regarding the agrobiological and technological characterization. Each phenophase was considered for registration when the dates were recorded at which 50% of the buds, flowers, grapes reached the respective phenological stages (BBCH 08 - budburst; BBCH 65 - flowering; BBCH 81 - veraison and BBCH 89 - ripe berries for harvesting). Phenological information was expressed as "day of year" (DOY), as the number of days after January 1. During the vegetation period, determinations were made on the basis of which the fertility coefficients (absolute, relative) and productivity indices (absolute and relative) were calculated, as well as the number of grapes per bunch, the average weight of a grape, the berry weight (g), yield (kg/vine), sugars (g/l), acidity (g/l tartaric acid), pH. The content of solide solubile totale (TSS), were determined from 5 grape for each sample with refractive device Kruss DR301-95 (% Brix) (Mureşan, 2014; Oltenacu, 2015).The titratable acidity (TA) was determined by titration with 0.1N NaOH to pH 8.1, and the results were expressed in g tartaric acid/100 g. Titratable acidity calculation was done using the formula: Titratable acidity (%) = (V x N x C x 100)/m where V = volume of NaOH consumed; N = NaOH normality; C = tartaric acid equivalent; m = sample mass; C has values: 0.0075 to express acidity in tartaric acid (specificity for grapes, shoots, bananas). The maturation index, also known as the acidometric balance index, was calculated using the formula: TSS/TA. For all determinations were performed in triplicate. Statistical analyses were performed using Excel, including: average, standard deviation. To evaluate the quality of the production obtained, the notion of grape production quality was quantified (Pârcălabu, 2010, cited by Stroe

& Budescu, 2013) which was expressed by three components: yield/vine, (kg/vine); TSS, (g/l); TA, (g/l tartaric acid). Therefore, the quality denoted by Q would be a vector with three elements: Q = (Y, TSS, TA) and which offers a perspective on the balance between quantity and quality which in table varieties are not in antithesis, but because the study also addresses the adaptability of the variety, this approach was needed. For this, each quality component (Y, TSS, TA) is divided by the optimal values of each variety: Yopt, TSSopt, TAopt, considered to be the values obtained in Italy, as follows: TSS: 14-15 (% Brix), TA: 4.5 (g/l tartaric acid) and yield 13.20 (kg/vine). Therefore, the following are defined: The production quality coefficient: cY defined by the equation: cY=Y/Yopt; The quality coefficient in the content of the must in sugars: cTSS defined by the equation: cTSS = TSS/TSSopt; The quality coefficient in the content of the must in total acidity: cTA=TA/TAopt. The quality vector has the component Q = (cY, cTSS, cTA). The best quality is considered when for each component, the quality coefficients have a value close to 1. his being the ideal case, c= (1, 1, 1) or if expressed as a percentage then this value will become c=(100%, 100%, 100%). If the values of the quality coefficients are sub-unit or super-unit, the quality of the harvest is not within optimal parameters. In order to more easily estimate how qualitatively a variety performs within an area or following an applied technology, the relative quality coefficient (relative to optimal values) can be introduced, also defined by the three components:

Relative quality coefficient in production: cY defined by the equation: cYr=(Y-Yopt)/Yopt=cY-1; Relative quality coefficient in the sugar content of the must: cTSSr = (TSS-TSSopt)/TSSopt = cTSS-1; The relative quality coefficient in the must content in total acidity defined by the equation: cTAr = (TA-TAopt)/TAopt = cTA-1. The quality vector has in this case the component Qr = (cYr, cTSSr, cTAr). When assessing the quality potential of a variety according to the relative quality coefficient, it will be taken into account that the variety has better adaptability to the area as the values of the relative quality coefficients approach zero.

RESULTS AND DISCUSSIONS

Climatic conditions

The analysis of the climatic context in the analyzed period (2022-2024), assessed by comparison with the average of the 1991-2020 interval, shows that thermal resources are very high compared to the average, both in relation to the multiannual average and for each individual year, against the backdrop of drastically reduced water resources. Total precipitation in the growing season (IV-X), in 2023 it decreased by 213 compared to the average mm, and in 2024 it records a decrease of 91 mm.

Table 1. The main climatic parameters and bioclimatic indices during the experimentation period

Climatic parameters and bioclimatic indices	Average (1991-2020)	Years	
		2023	2024
Average annual temperature, ºC	10.98	14.61	14.70
Average temperature in the growing season, ºC (IV-X)	17.46	21.01	20.62
Average temperature in summer, ºC (VI-VIII)	22.15	27.22	26.30
Average annual minimum temperature, ºC	5.24	7.10	7.70
Average annual maximum temperature, ºC	17.6	21.20	21.10
Number of hot days (T_{max} > 30°C)	48	54	52
Number of very hot days (T_{max} > 35°C)	10	23	38
Annual total precipitation, mm	633	435	583
Total precipitation in the growing season (IV-X), mm	430	217	339
Total precipitation in summer (VI-VIII), mm	193	94	180
Hydrothermal coefficient (CH)	1.1	0.64	0.75
Huglin index (HI, Huglin, 1978)	3163	4054	4138
Winkler index (WI, Winkler)	1762	2356	2273
Cool night index (CI)	10.64	14.1	13.5

But even in these conditions, at such values, good results cannot be obtained, regardless of the variety or the set of green works and operations performed, irrigation being needed especially in critical phenophases. As for the climatic indicators (Huglin index, Winkler index, Cool night index), their values register a large amplitude, far exceeding the limits of a warm temperate climate with cool nights and passing into a warm climate with cold nights, (Bucur & Stroe, 2023). Practically, average temperature in summer, ºC (VI-VIII) (27.22 and 26.30) and especially those recorded in the last decade of ripening, accompanied by the temperatures recorded during the night (with differences from day to night) leave their mark on TSS (%), TA, gluco-acidometric index, pH

which are at the limit of potential according to the data in the genetic passport of the variety (Table 1).

Development of the main phenophases

The onset of the phenological stages and the interval between them are given by the genetics of the variety, first of all, then by the climatic and soil conditions and viticultural practices. For both years taken into study (Table 2) the average day of the year (DOY) values of the four phenological stages of the vine (budburst, flowering, veraison and harvest) show that, budbreak occurred on average 106 DOY (April 16 - 2023) and (April 102 - 2024). **Flowering** occurred on average at DOY 161 (June 10), exactly 55 days after budbreak (Stroe, 2020) suggesting precocity in this area of southern Romania as well. Regarding the other phenophases **veraison** was recorded on average at DOY 218, respectively 217 (August 6, 2023 and August 5, 2024), demonstrating a constancy of the variety. The average day of the year of ripening grape, for the 2 years studied, was DOY 246 and 248 (September 03 and September 5), with differences of only 2 days. Looking at the climatic conditions - average temperature in the growing season, ºC (IV-X) records higher values in 2023 (21.01), which shows that the onset of an earlier bud break in 2023 was followed by a close approximation of the data for the other 3 phenophases.

Fertility and productivity

The elements of fertility and productivity represent a complex determinant of the level of production that are influenced by the hereditary basis, the rootstock used, the applied agrotechnics, as well as the particularities of the climatic conditions specific to each harvest year. According to the data recorded in Tables 2 and 3, the variety was distinguished by an average fertility, falling into the variation class 1.1-2.0. The highest value was obtained in 2024 - (Fca = 1.21, Fcr = 0.75). These values influenced the productivity indices values, which due to the large size of the average weight of the grape (525.4 g), had values ranging between 635.73 g/shoot (Pia), respectively 394.05 g/ shoot (Pir); Finally, the number of grapes obtained per vine (Table 3), together with the average weight, gives us a perspective of the total production per vine - 11.02 kg/vine (2023) and 12.08 kg/vine (2024), quite high values for the specific conditions of the southeast of Romania. A positive side can be noted in the case of berry weight where the average value recorded of 5.35g although small compared to the potential of the variety (6-7 g) is large enough to ensure a special commercial appearance to the bunch. Regarding the organoleptic potential seen from the perspective of variation of: TSS (%), TA, gluco-acidometric index, pH during the 2022-2024, these fully meet the quality standards of table grape varieties, according to the commercial quality standards developed by the Working Party on Agricultural Quality Standards of the United Nations Economic Commission for Europe (UNECE, 2017) and OIV Resolution VITI 1/2008 (OIV, 2008, cited by Cichi et al., 2023; Stroe et al., 2021). Table grapes can be harvested at lower sugar concentrations compared to wine varieties, but not less than 16°Brix, with the exception of a few exceptions depending on the climate of each country. The data referring to these parameters indicate values that prove that the 'Matilde' variety has qualities that suggest that it can sometimes surpass its parental phenotype (especially the 'Cardinal' variety). The amounts of accumulated sugars are satisfactory, being in correlation with acidity, ensuring a balanced gluco-acidimetric index in accordance with standards required by the consumer (2.5÷4.5).

http://catalogoviti.politicheagricole.it/scheda.php?codice=547

Table 2. Mean day of year (DOY) of the phenological stages (budburst; flowering; veraison and harvest) and the synthesis of the main fertility elements of varieties study (2022-2024)

Main phenophases	Budburst date (DOY)	Flowering date (DOY)	Veraison date (DOY)	Harvest maturity date (DOY)
	Mean	Mean	Mean	Mean
2023	106 (April 16)	161 (June 10)	218 (August 6)	246 (September 3)
2024	102 (April 12)	161 (june 10)	217 (August5)	248 (September 5)

	Fertility coefficient (Fc)		Bunch weight (g)	Productivity indices (Pi)		Y/v[1] (kg/vine)	Y/ha (kg estimated)
	absolute	relative		Absolute (g/shoot)	Relative (g/shoot)		
2023	1.10	0.66	509.4	560.34	336.2	11.20	42.414
2024	1.21	0.75	525.4	635.73	394.05	12.08	45.747
					Mean	**11.64**	**44.080**

[1]Y/v = Yield/vine (kg)

Table 3.Synthesis regarding the characteristics of grapes and berries of the studied variety during the 2022-2024

Experimental year	No. bunch/vine		Bunch weight (g)		Berry weight (g)	
	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
2022-2023	22.0	±3.39	509.4	±12.92	5.3	±0.40
2023-2024	28.0	±3.74	525.4	±100.19	5.4	±0.25
Mean	25	±3.56	517.7	±11.67	5.35	±0.32

Table 4. Variation of: TSS (%), TA, gluco-acidometric index, pH during the 2022-2024

Experimental year	TSS[2](%)		TA[3] (g/L tartaric acid)		Gluco-acidometric index		pH	
	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
2022-2023	16.1	±0.60	4.00	±0.10	3.70	±0.35	3.51	±0.07
2023-2024	15.0	±0.12	3.9	±0.36	3.48	±0.24	3.63	±0.11
Mean	15.55	±0.36	3.95	±0.23	3.59	±0.29	3.57	±0.09

[2]TSS% = total soluble solids (Brix);[3]TA% Titrable acidity

Table 5. Variation of: TSS (%), TA, gluco-acidometric index, pH during the 2022-2023

Mean experimental years - (2022-2023); (2023-2024)				
'M A T I L D E'	c*Y* =Y/Y_{opt}[4] 0.88	Q = (cY, cTSS, cTA) (0.88; 1.07; 0.87) (88%, 107.24%, 87%)	*cYr =(Y-Yopt)/Yopt= cY -1* 0.12	Qr = (c*Yr*, c*TSSr*, c*TAr*) (0.12; 0.072; 0.13)
	c*TSS* =TSS/TSS_{opt}[4] 1.07		*cTSSr =(TSS-TSSopt)/TSSopt = cTSS -1* 0.072	
	c*TA* =TA/TA_{opt}[4] 0.87		*cTAr = (TA-TAopt)/TAopt = cTA -1* 0.13	

[4]The optimal values are: sugars 14.5[0] Brix (TSS), 4.5 (g/l tartaric acid) TA; 13.20 (kg/vine) Y/v[1]

The TSS(%), TA (g/L tartaric acid) values, which are a good substrate for the entire metabolism of the plant, emphasize the discrete muscat aroma of the variety (from the maternal parent 'Italia' variety). Regarding the evaluation of the productive balance in terms of defining quality parameters, this was expressed by three components: yield/vine, (kg/vine); TSS, (g/l); TA, (g/l tartaric acid) and the following were obtained: for the production parameter, respectively the acidity of the fresh product, the values obtained 0.88 or 88% and 0.87 (87%) indicate that from this point of view the variety was below the optimal threshold. The value of 1.07 is the closest to the value 1 (ideal), for the sugars accumulated in the berries, which indicates that the variety has reached a technological potential very close to the optimum (varietal potential) at the moment of full maturity. The assessment of the

qualitative potential of the variety according to the values of the relative quality coefficient emphasizes the same thing that, in terms of the accumulation of sugars reached, the variety registers values close to zero, 0.04, which indicates a good adaptation of the variety against the backdrop of very favorable ecopedoclimatic conditions, followed hierarchically by acidity and yied/vine, whose values are lower, but quite high for the specific conditions of the Southeast of Romania.

CONCLUSIONS

The behavior of the 'Matilde' variety in an area quite favorable for the cultivation of vines, (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m), related to the objectives of the study - demonstrates a good adaptability. The data indicating the agrobiological and technological potential prove that the 'Matilde' variety has qualities that suggest that it can sometimes surpass its parental phenotype (especially the 'Cardinal' variety). The special organoleptic potential of the variety, marked by the quantities of accumulated sugars in good balance with acidity, ensures a balanced gluco-acidimetric index, located within the preferential limits of consumers. The results obtained, verified by calculating specific and representative indices and supported by the attributes and quality of the production, offer a positive perspective, which can include the variety in the planting list of economic agents who grow table grapes.

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ALTERNATIVE APPROACHES FOR CONTROLLING SOME DISEASES AND PESTS IN GRAPEVINES

Diana Elena VIZITIU[1], Ionuț ȘANDRIC[2], Anamaria DUMITRU[1], Dorin Ioan SUMEDREA[1], Adrian NIȚU[3], Cristina MIHĂESCU[4], Ionela-Daniela SĂRDĂRESCU[1]

[1]National Research and Development Institute for Biotechnology in Horticulture Ștefănești - Argeş, 117715, Romania
[2]University of Bucharest, Bucharest, 010041, Romania
[3]KWIZDA Agro Romania, 93-95 Emanoil Porumbaru Street, District 1, Bucharest, Romania
[4]National University of Science and Technology POLITEHNICA Bucharest, Pitești University Centre, Romania

Corresponding author email: anamaria.ilina@yahoo.com

Abstract

The research aimed to test the behavior of the Argessis variety in the organic farming system under the conditions of the Ștefănești Viticultural Center. The study included monitoring the evolution of diseases and pests (Plasmopara viticola, Uncinula necator, Guignardia bidwellii, Eriophyes vitis) during 2024, application of a treatment program adapted to the moment of appearance and intensity of the attacks, as well as evaluating some agrobiological indicators (grapes weight, berry weight, trunk production, sugar content, and must acidity). The results showed that the attacks of mites and downy mildew stopped completely in June after applying treatments with Limocide and Copperfield. In July, black rot settled on the grapes, but was controlled by increasing the dose of Copperfield; the pathogens were no longer present until harvest. Regarding production parameters, the Argessis variety recorded an average grape weight above the values in the specialized literature. The sugar and must acidity levels were within limits close to those reported in the specialized literature. The study demonstrates the efficiency of alternative products to chemical pesticides for ensuring grapevine health and maintaining competitive production parameters.

Key words*: downy mildew, eriophyid mites, black rot of grapevine, Vitis vinifera, grapevine.*

INTRODUCTION

Pesticides play a crucial role in modern agriculture by safeguarding crops from pests and diseases, ensuring food safety. Nevertheless, the use of pesticides in food production has sparked considerable concerns about their potential effects on human health and the environment (Lazarević-Pašti et al., 2025). To be beneficial to human health and the environment, they must be used in moderation and with a full understanding of their categorisation (Pathak et al., 2022).

In the European Union, the number of pollinators has declined significantly in recent decades, with one in three bee and butterfly species in decline, and one in ten such species on the verge of extinction. Pollinators are essential for the functioning of terrestrial ecosystems, human welfare, and food security, as they facilitate the pollination of both wild and cultivated plants. Almost EUR 5.000.000.000 of the Union's annual agricultural production is directly attributable to pollinating insects. Sustainable, resilient and biodiversity-rich agricultural ecosystems are therefore needed to ensure safe, nutritious, and affordable food. Agricultural ecosystems rich in biodiversity increase agricultural resilience to climate change and environmental risks.

One of the practices that has significant multiple benefits for biodiversity protection, ecosystem services, and landscape elements is organic farming (EU Regulation 869, 2022).

Vitis vinifera L. is among the most commercially significant fruit species globally (Romero-Romero et al., 2025) and grapes rank among the most widely consumed fruits worldwide, primarily utilised in the production of juice and wine (Haseeb et al., 2019; Martin et al., 2020).

Pesticides are used in grapevine culture to control pests and diseases (Flamini, 2003). These can have negative impacts on human health and the environment (Metral et al., 2024). Grapevines are susceptible to a range of phytopathogenic diseases throughout their

developmental cycle, including downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), black rot (*Guignardia bidwellii*), and anthracnose (*Elsinoe ampelina*). The diseases and pests can significantly compromise both the yield and quality of the fruit. In intensive viticultural systems, applying chemical pesticides remains a primary strategy for crop protection, many pathogens and pests can cause severe reductions in productivity and agricultural output (Alsufyani et al., 2024; Syrgabek et al., 2023; Tukenova et al., 2021).

In grape cultivation, the improper or excessive use of pesticides can lead to residue levels that surpass established safety thresholds. These pesticide residues not only pose risks to environmental health but may also compromise the quality of grapes and their derived products, potentially exerting adverse effects on human health (Syrgabek et al., 2022).

Practising organic farming also has a positive impact on the climate (EU Biodiversity Strategy for 2030, 2020), encourages biodiversity, natural biological cycles, and enhances product quality (Dina et al., 2024).

The purpose of this study was to develop a treatment schedule and evaluate the effect of organic products on diseases, pests, and the yield of grapevine plants, providing an alternative to chemical control.

MATERIALS AND METHODS

The study took place at the National Research and Development Institute for Biotechnologies in Horticulture Ștefănești - Argeș (INCDBH Ștefănești), in a table grape vineyard with the Argessis variety (0.5 ha). The distance between rows is 2.5 m, and between plants/row is 1 m. At the beginning of the vegetation period, a treatment scheme was developed, which was modified throughout the year depending on the appearance and intensity of the attack of grapevine plants' pathogens and pests (Table 1): *P. viticola*, *U. necator*, *G. bidwellii*, and *Eriophyes vitis* (erineum leaf mite).

Throughout the vegetation period, in 2024, the attack of harmful organisms was monitored, and the following products were applied: Flosul (*U. necator*), Limocide (*P. viticola, Eriophyes vitis*), Vitisan (*U. necator, B. cinerea*), Copperfield (*P. viticola, G. bidwelli*), Wuxal ascofol (biostimulant based on seaweed), Wuxal amino (liquid biostimulator based on organic nitrogen), Amargerol essence (organic biostimulator).

Table 1 Treatment schedule

No.	Date	Product and dosage (for 0.5 ha)
1.	04/16/2024	Flosul 1.5 L Limocide 1 L
2.	04/29/2024	Flosul 1.5 L Limocide 1 L Vitisan 2 kg
3.	05/08/2024	Flosul 1.5 L Vitisan 2 kg Copperfield 1 L Wuxal ascofol 1.5 L
4.	05/27/2024	Flosul 2 L Vitisan 2 kg Copperfield 1 L Wuxal amino 1.5 L
5.	06/11/2024	Flosul 2 L Vitisan 2 kg Copperfield 1 L Wuxal amino 1.5 L
6.	06/21/2024	Flosul 2 L Vitisan 2 kg Copperfield 1 L Amargerol 1.5 L
7.	07/05/2024	Flosul 2 L Vitisan 2 kg Copperfield 1 L
8.	07/16/2024	Flosul 2 L Vitisan 2 kg Copperfield 1.25 L Wuxal amino 1.5 L
9.	07/21/2024	Copperfield 1.25 L
10.	07/29/2024	Flosul 2 L Vitisan 2 kg Copperfield 1.25 L Wuxal amino 1.5 L
11.	08/09/2024	Flosul 2 L Vitisan 2 kg Copperfield 1.25 L Wuxal amino 1.5 L

Eriophyid mites and downy mildew produced symptoms only on leaves, and black rot only on grapes. To monitor their attack, the frequency (F), intensity (I), and degree of attack (GA) were determined (Vizitiu et al., 2022).

In order to find out the behaviour of the Argessis variety in an ecological culture, the main agrobiological characteristics were also determined: grape weight, berry weight, the grape production per m^2, sugar, and total acidity

of the must. Sugar and acidity were measured with the PAL-BRIACID2 device.
To quantify how the attack of mites and downy mildew evolves after each treatment, the daily means (eq. 1) were compared to a baseline set at the treatment date.

$$m_{p,t,k} = mean\ of\ metric\ k \in \{I, F, GA\} for\ pest\ p\ on\ date\ t$$

Because measurements occur on 08.05.2024, 16.05.2024 and 30.05.2024, two complementary, pre-specified baseline strategies were used:

- Nearest-after (strict): the baseline $b_{p.k.j}$ is the first measurement on or after the treatment date. This is conservative, but for Tx2 (27.05.2024), it sets the baseline at 30.05.2024, which prevents detecting a decrease between 27.05.2024 and 30.05.2024.
- Nearest-before (practical for sparse sampling): the baseline is the last measurement on or before. For Tx2, this uses 16.05.2024 as baseline, allowing the 27.05.2024 → 30.05.2024 change to be quantified.

The first decrease date was calculated according to eq 2:

$$d_{p,k,j} = \min\{t > t_0 : m_{p,t,k} < b_{p,k,j}$$

where t_0 is the baseline date determined by the algorithm.

The attack is considered stopped when the mean meets a predefined threshold θ (eq 3). The value of 0 was used by default; a small $\varepsilon > 0$ can be substituted to define "near-zero" if desired.

$$S_{p,k,j} = \min\{t > \tau_j : m_{p,t,k} \leq \theta\}$$

To assess the efficiency of the treatment for black rot, the Mann-Whitney test was applied by comparing the pre- and post-treatments of I, F, and GA. Before any between-group comparisons, normality was checked (Shapiro–Wilk).
The statistical analysis was implemented using the Python programming language and the following modules: pandas, scikit-learn, and matplotlib.

RESULTS AND DISCUSSIONS

Insect pests inflict substantial damage on global agriculture, leading to significant financial and environmental repercussions (Yactayo-Chang et al., 2020).
Agricultural expansion and intensification at the global level have led to the decline of ecological infrastructures essential for insects, herbaceous plants, and other organisms. Therefore, agriculture is considered a major factor in the biodiversity decline, and initiatives such as the European Green Deal highlight the importance of reducing ecosystem degradation. Among fruit crops, grapevine culture has one of the most intensive agricultural systems with the highest economic importance (Marcelino et al., 2024).
In 2024, eriophyid mites (Figure 1) and downy mildew (Figure 2) first appeared in May. The eriophyid mites attack was stronger than the downy mildew; the latter usually manifested itself by the appearance of 1-2 spots on the upper surface of the leaves, rarely accompanied by fruiting on the underside of the leaves. Nymphs and adults of *E. vitis* settled on the underside of the leaves and fed on their sap, which led to the appearance of swellings on the upper side of the leaves.



Figure 1. Galls produced by eriophyid mites (05/07/2024)

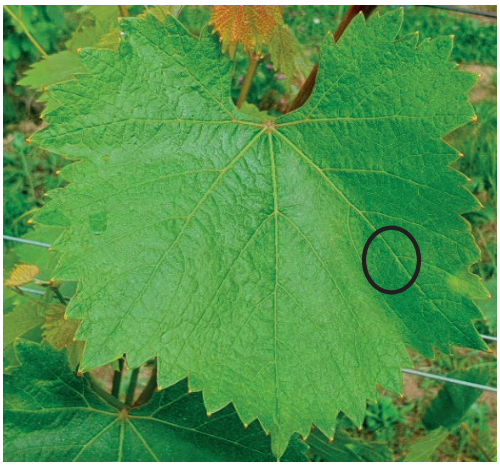


Figure 2. The appearance of the first downy mildew spots

E. vitis is a pest with significant potential to affect plant physiological performance and production quality. Monitoring the attack dynamics by assessing the symptoms frequency and intensity is a fundamental tool for understanding the establishment and evolution of populations on the leaf. The integration of these parameters in the attack degree indicator allows a more precise characterization of the severity of the infestation and the effectiveness of control measures.

The frequency of attack showed a progressive increase in the first half of May, suggesting a rapid establishment of *E. vitis* colonies on the leaves. Subsequently, after applying the treatments on May 8, 2024, and May 27, 2024, a visible decrease in frequency was recorded, indicating the organic products' effectiveness in limiting the spread of the pest (Figure 3).

The intensity of the attack followed the same trend as the frequency: an initial increase, reaching a peak in mid-May, followed by a significant reduction after the application of successive treatments. This dynamic confirms that the interventions were able to reduce not only the number of affected plants, but also the severity of the symptoms (Figure 4).

The degree of attack faithfully reflected the evolution of the other two parameters: a steady increase until severe symptoms appeared, followed by a clear decline after treatments. The data suggest that the applied scheme was able to stop the progression of the attack before it caused economic damage (Figure 5).

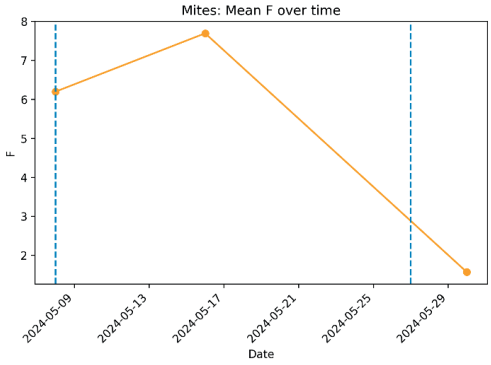


Figure 3. Evolution of the eriophyid mites frequency attacks in May 2024

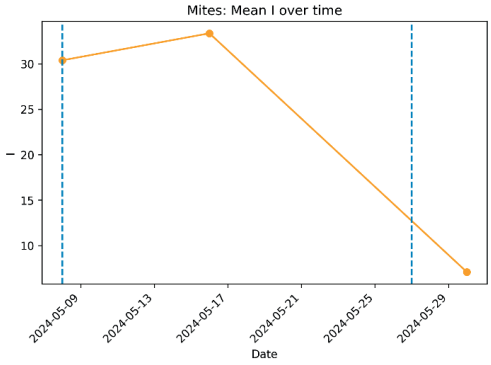


Figure 4. Evolution of the eriophyid mites intensity attacks in May 2024

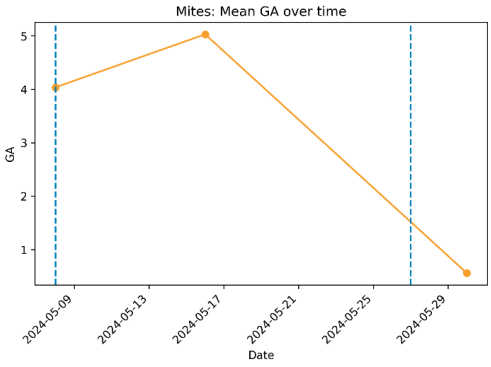


Figure 5. Evolution of the eriophyid mites degree of attacks in May 2024

Downy mildew is a disease that reduces grapevine yield (Voiculescu et al., 2021). Therefore, monitoring the attack evolution caused by *P. viticola* is essential for understanding the infection pressure and for substantiating protection strategies. The analysis

of the frequency and intensity of symptoms, as well as the degree of attack, made it possible to capture both the dynamics of disease occurrence and the severity of leaf manifestations, providing a comprehensive picture of infection evolution and treatment effectiveness.

At the beginning of May (May 9, 2024), the frequency was 0.19%, but the infection spread rapidly until May 17, 2024, when it reached 0.26%. After this date, the frequency of *P. viticola* infection decreased steadily to 0.13% (May 29, 2024), indicating a reduction in pathogen spread (Figure 6).

The intensity of downy mildew showed high values at the beginning of May (4.2%), remaining at the same level until May 17, 2024. In the following period, a sharp decrease was observed, reaching 1.2% by the end of the month (May 29, 2024), which reflects a considerable reduction in symptom severity in the vineyard plantation (Figure 7).

The values of the degree of attack remained relatively constant in the first half of May (0.041%), but after May 17, 2024, they recorded a significant decline, reaching 0.011% on May 29, 2024. This trend suggests a reduction in the epidemiological pressure exerted by *P. viticola* in the second part of May (Figure 8).

These results indicate that the applied organic products limited the development of primary infections and prevented the occurrence of secondary ones, ensuring adequate protection of the grape and grapevine canopy.

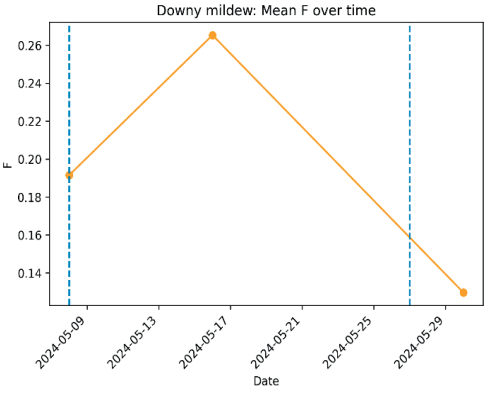


Figure 6. Evolution of downy mildew frequency attack in May 2024

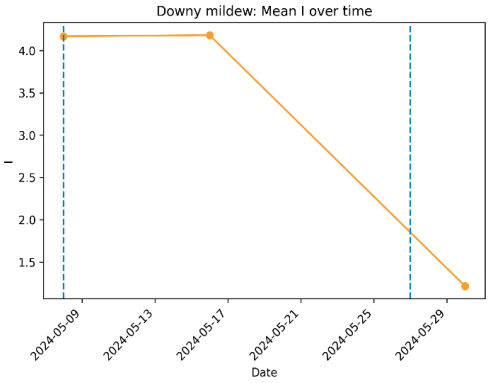


Figure 7. Evolution of downy mildew intensity attack in May 2024

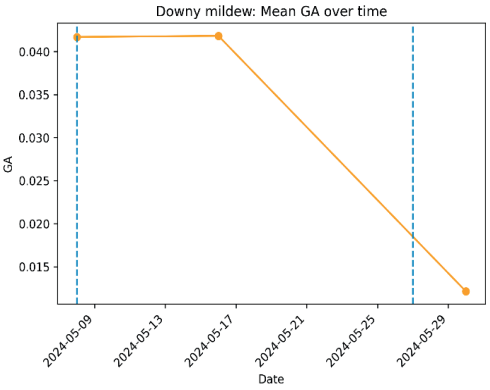


Figure 8. Evolution of downy mildew frequency attack in May 2024

From the beginning of June until the end of the growing season, mites and downy mildew were no longer present in the vineyard.

In July, black rot appeared on the grapes (Figure 9). After the appearance of this pathogen, the Copperfield dose was increased from 1.0 l/0.5 ha to 1.25 l/0.5 ha. To monitor this pathogen, the frequency, intensity, and degree of attack were determined before increasing the Copperfield product dose (July 15, 2024), but also after the application of treatments with this increased dose (July 28, 2024). Increasing the Copperfield product dose led to the attack stopping after the second treatment. Since the Shapiro-Wilk test showed that the data were not normally distributed, the Mann-Whitney U test (a non-parametric alternative to the t-test) was used.

Thus, after interpreting the data using the Mann-Whitney U test, it was found that, monitoring the same grapes, no significant differences between the determinations in terms of frequency, intensity, and degree of attack of the black rot were observed between 12.07.2025 and 27.07.2025 (Table 2). Black rot was monitored until the grapes were harvested, during which time the absence of pathogen-specific manifestations was noted. Therefore, the increased dose of the Copperfield product stopped the attack of black rot.
No other grapevine diseases were present on the grapevine plants until grape harvest.

Table 2. Statistical analysis of the black rot evolution on Argessis grapes

F	I	GA
Mann-Whitney value = 837.500, p = 0.455	Mann-Whitney value = 1010.000, p = 0.460	Mann-Whitney value = 914.500, p = 0.935



Figure 9. Argessis cultivar affected by black rot (July 2024)

Black rot is among the most difficult diseases to control in organic vineyards, as *G. bidwellii* shows low sensitivity to copper-based treatments (Jones & McManus, 2017).
Alternative products such as Limoncide (based on citrus extracts), Flosul (based on micronized sulfur), and Vitisan (potassium hydrogen carbonate) are increasingly used in organic agriculture and viticulture due to their preventive and curative action against fungal pathogens, offering a sustainable option to reduce the use of conventional synthetic fungicides. For example, Limocide has demonstrated high efficacy against the fungi responsible for crown rot in dessert bananas (Kouame et al., 2025); Flosul can be used to control grapevine powdery mildew (Wicks et al., 2003); Vitisan, applied together with Bas foliar Aktiv, had a satisfactory effect against grape powdery mildew (Žežlina et al., 2010).
The climate data for the 2023-2024 wine-growing period (Figure 10) show significant deviations from the multiannual average (1979–2018). The average monthly temperature recorded higher values almost throughout the year, with important positive deviations in February (+5.62°C) and in the June-August period (+2.76-3.55°C). These differences confirm the climate warming trend reported in other European wine-growing regions, where acceleration of phenophases, reduction of ripening time, and possible imbalances between technological and phenolic ripening of grapes were observed (Jones et al., 2005; van Leeuwen & Darriet, 2016).
The rainfall regime showed high variability, with surpluses in November 2023 (+42.92%) and September 2024 (+149.00%), but with accentuated deficits in May (-73.73%), June (-79.04%), July (-48.18%) and August (-89.45%). This uneven distribution of precipitation is characteristic of the intensification of extreme climatic phenomena (Schultz, 2016) and can generate risks of water stress during the critical period of berries development, but also excess water in other stages, with possible consequences on the quality of production and on the pressure of cryptogamic diseases (Palliotti et al., 2014).

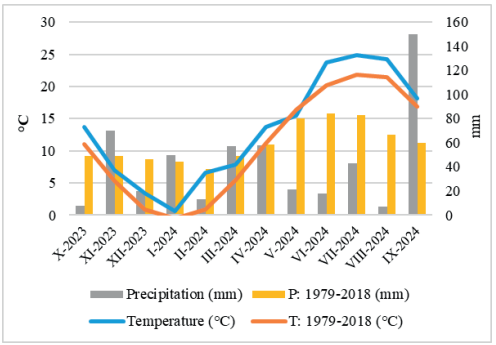


Figure 10. Evolution of the main climatic factors

Overall, the results confirm the major impact of climate change on viticulture, highlighting the need to adapt technologies through more efficient management of water resources (localized irrigation, mulching), the use of varieties and rootstocks resistant to abiotic stress, and adjusting the calendar of green works, as recommended by other researchers (Ollat et al., 2017; Santos et al., 2020).
In addition to the fungicidal and insecticidal activity, the following agrobiological indicators were also determined: grape weight, berry weight, grape production per m^2, sugar amount from the must, and total acidity of the must (H2SO4). In order to carry out these determinations, the grapes were harvested (Figure 11) and processed on 25.09.2024.



Figure 11. Argessis grapes at harvest time (25.09.2024)

Argessis table grapes showed a very good evolution (Figure 9), averaging 491.31 g, which is 18.67% higher than the values found in the specialised literature. However, the average weight of a berry was 5.34 g, representing 17.44% less than the values from the specialised literature (Table 3).

Table 3. Main agrobiological characteristics of the Argessis variety

Descriptor	Average in the demonstration plot	Average after Glăman et al., 2018*
Grape weight	491.31 g	414 g
Berry weight	5.34 g	6.5 g
Grape production per m^2	4.78 kg/trunk	-
Sugar amount from the must	146.67 g/l	150 g/l
Total acidity of the must (H2SO4)	3.43 g/l	3.8 g/L

CONCLUSIONS

The organic products and biostimulants tested (Flosul, Limocide, Vitisan, Copperfield, Wuxal, Amargerol) were highly effective in controlling mites, downy mildew, and black rot.
Mites and downy mildew had a stronger spread in the first half of May, but their attack was stopped due to the ecological products applied.
Increasing the dose of Copperfield led to the complete stoppage of the black rot attack, confirmed by monitoring during the growing season and by the statistical tests applied.
Argessis cultivar has demonstrated good tolerance to the main diseases and, as a result, good adaptation to the organic farming system, with satisfactory production and quality parameters comparable to data from the specialized literature.
The results highlight the potential of using alternative products to chemical pesticides in viticulture, thereby contributing to reducing the environmental impact and improving the sustainability of agriculture.

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VEGETABLE GROWING


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COMPARATIVE STUDY OF THE NUTRITIONAL COMPOSITION OF MAJOR LEGUME SPECIES

Ovidia Loredana AGAPIE, Elena BARCANU TUDOR, Ion GHERASE, Eliza TEODORESCU

Vegetable Research and Development Station Buzău, 23rd Mesteacănului Street, 120024, Buzău, Romania

Corresponding author email: teodorescu.eliza@gmail.com

Abstract

Legumes are essential in traditional diets, providing nutritional and health benefits, while also supporting economic stability and environmental sustainability. This study evaluates the nutritional quality of three legume species: Vicia faba L., Pisum sativum L., and Phaseolus vulgaris L., by analysing total dry matter, total soluble solids (TSS) and titratable acidity. These parameters were measured using standardized methodologies on selected genotypes cultivated under controlled conditions. Significant inter- and intra-species variability was observed. Broad bean genotypes, especially L5, exhibited the highest dry matter content (34.55%), which is often associated with increased concentrations of essential nutrients. Pea genotypes, such as L6, demonstrated elevated TSS levels (>14°Brix), suggesting potential for fresh market applications. Common bean genotypes, particularly the climbing variety L135, showed high TSS values (16.03°Brix) and moderate acidity, underscoring their suitability for processing. These results emphasize the importance of genetic diversity in legumes to optimize nutritional value and processing potential. This study supports the development of targeted cultivation and breeding strategies to improve legume productivity and quality under diverse environmental conditions, particularly in the context of climate change.

Key words*: legumes, nutritional quality, Vicia faba L., Pisum sativum L., Phaseolus vulgaris L.*

INTRODUCTION

Legumes belonging to the Fabaceae family, have been a cornerstone of human nutrition since ancient times, with their seeds playing a vital role in sustaining diets across cultures (Martín-Cabrejas, 2019).

Nowadays, legumes are highly valued for their exceptional nutritional benefits and associated health advantages (Trinidad et al., 2010), as well as their vital economic and environmental roles in promoting a sustainable future (Reckling et al., 2016; Brezeanu et al., 2021).

Common legumes used for human consumption include peas, broad beans, lentils, soybeans, lotus, lupins, mung bean, sprouts, green beans and peanuts and are referred to as food legumes or grain legumes and they are classified into two categories: oil seeds and pulses (Maphosa & Jideani, 2017).

Legume crops play a vital role in sustainable agriculture and have the ability to adapt to climate change, thriving in arid regions with scarce or unpredictable rainfall (Duc et al., 2015; Stagnari et al., 2017). In addition, legumes are widely recognized for their unique ability to fix atmospheric nitrogen through a symbiotic relationship with rhizobia bacteria, which form nodules on their roots. This process, called biological nitrogen fixation (BNF), transforms atmospheric nitrogen into a plant-usable form, boosting soil fertility and minimizing reliance on chemical fertilizers, resulting in reduced greenhouse gas emissions (Kebede, 2021).

Also, legumes serve as diversification crops in agroecosystems dominated by a few major species, helping to disrupt pest and disease cycles while addressing the plant protein production deficit in many regions worldwide, including Europe (Peoples et al., 2009; Stagnari et al., 2017).

The consumption of legumes, including lentils, chickpeas, black beans, and soybeans, has been reported to be associated with numerous beneficial health attributes, such as antiatherogenic, hypocholesterolemic, hypoglycemic, and anticarcinogenic properties (Ndidi et al., 2014; Messina, 2019).

The nutritional value of legumes is attracting significant attention in developed countries due to the growing demand for healthy foods. They are rich in proteins, carbohydrates, and dietary fibres, as well as other essential nutrients. (Bouchenak et al., 2013). With increasing global demand for nutrient-dense food, legumes like broad bean (*Vicia faba* L.), pea (*Pisum sativum* L.), and common bean (*Phaseolus vulgaris* L.) are recognized as vital components of a balanced diet (Dhull et al., 2022).

This study aims to evaluate the nutritional quality of Romanian selected legume genotypes (pea, broad bean and common bean) by examining key parameters: total dry matter (indicator of nutrient density), total soluble solids (related to energy value and taste), and titratable acidity (important for processing and flavour). By analysing these traits across species and genotypes, this research highlights the genetic diversity and adaptability of legumes for nutritional and industrial applications.

MATERIALS AND METHODS

The study analysed a total of 13 broad bean accessions (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L17, L21, L22), 15 pea accessions (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15), 8 dwarf bean accessions (L1, L4, L5, L34, L35, L36, L39, L40), and 6 climbing bean accessions (L104, L112, L115, L125, L136, L141). These local cultivars were either gathered from various regions of Romania or developed through segregation and selection processes.

The experiment was conducted in the year 2024 at the experimental plot of the Vegetable Research and Development Station Buzau (45.13711°N, 26.81711°E and 95 m a.s.l.) in Buzau County, Romania. The crop management practices employed were specific to *Vicia faba* L., *Pisum sativum* L., and *Phaseolus vulgaris* L., as previously detailed in our earlier study (Tănase et al., 2020; Gherase et al., 2021; Barcanu et al., 2022).

Nutritional analysis was conducted to assess the following parameters: Total Dry Matter (DM), measured using the AOAC 930.04 method (Loss on Drying); Total Soluble Solids (TSS), determined with a KERN OPTICS refractometer and expressed in °Brix (AOAC 932.12); and Titratable Acidity (TA), evaluated with a TitroLine Easy titrator and expressed as a percentage of citric acid (AOAC 942.15).

Data analysis was performed using one-way ANOVA at a 95% confidence interval, followed by Duncan's test for multiple comparisons.

RESULTS AND DISCUSSIONS

Climatic data

In 2024, Buzău area experienced extreme climatic events, with the hottest summer on record (Figure 1), marked by intense heatwaves, record-breaking temperatures, and prolonged drought. These conditions alternated with short episodes of intense rainfall, highlighting the uneven distribution of precipitation. The significant increase in annual average temperatures and greater variability in precipitation reflect the impact of global climate change on the region. These changes pose challenges for agriculture, particularly vegetable farming, and the management of natural resources.

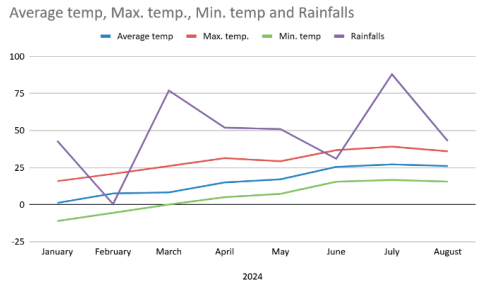


Figure 1. The meteorological data for the year 2024

Vicia faba L.

Phenological observations were also made for the broad bean accessions (data not shown). The results obtained demonstrate that the L2 accession exhibits the earliest development, followed by L3 and L5, whereas L6 and L17 are positioned at the opposite end of the spectrum, representing the latest maturing accessions.

The Total Soluble Solids (TSS) content in faba beans plays a crucial role for breeders focused on improving seed quality and for processors

looking to maximize the nutritional benefits of faba bean-derived products (Ahmed et al., 2022). Our results indicated that early varieties (L2-17.03°Brix, L8-18.09°Brix) had a higher TSS content compared to late varieties (L6-8.18°Brix, L4-8.7°Brix). Studies by Pinillos et al. (2011), also suggest that higher TSS values may be associated with earlier maturation of the accessions. The total soluble solids content ranged from 8.18°Brix (L7) to 18.09°Brix (L8) as shown in Figure 2.

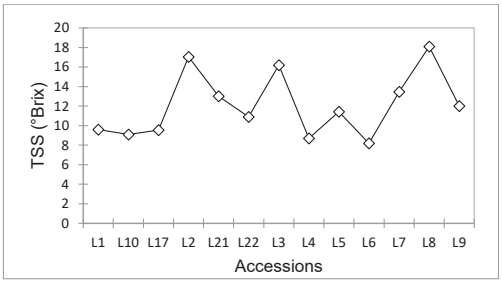


Figure 2. The mean values of TSS content of broad bean accessions

According to De Cillis et al. (2019), TA can be regarded as a key quality attribute for processing faba beans, as, similar to other vegetables, higher TA values help in better managing microbial spoilage in the final product. Acidity also affects the colour and overall stability of vegetables. Fruits and vegetables with low levels of organic acids may lack their characteristic flavour (Kader et al., 2008). For the analysed accessions (Figure 3), it was observed that L5 had the highest TA at 1.33% citric acid, while L8, at the opposite end, exhibited the lowest value, with only 0.47% citric acid.

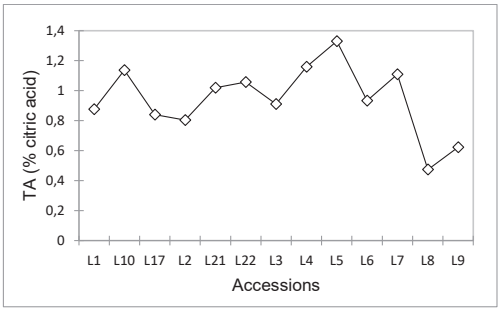


Figure 3. The mean values of TA content of broad bean accessions

The total dry matter content (Figure 4) ranged from 20.76% (L10) to 34.55% (L5). Additionally, a positive correlation (r = 0.58) was observed between dry matter content and grain weight. This suggests that larger and denser grains tend to have a higher dry matter content, which reflects a greater nutritional value.

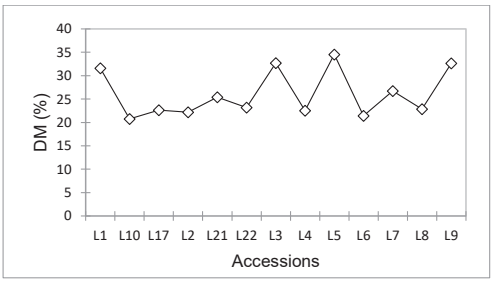


Figure 4. The mean values of DM content of broad bean accessions

***Pisum sativum* L.**

A fundamental quality parameter of peas is the total soluble solids (TSS) content, measured in degrees Brix (°Brix). Studies conducted by Nleya et al. (2014) and García-García et al. (2022), suggest that high-quality peas should have a hight sugar content to be classified as sweet. In this study, TSS content ranging from 14.54°Brix (L13) to 39.99°Brix (L11) (Figure 5), therefore, all the studied accessions can be classified as sweet varieties.

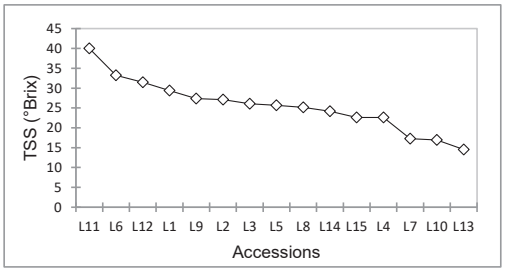


Figure 5. The mean values of TSS content of pea accessions

Total acidity affects the final flavour profile of peas, particularly when incorporated into formulations containing acidic ingredients. Peas are generally characterized by a low acidity level, which contributes to their high digestibility and suitability for inclusion in diets designed for individuals with gastric sensitivity (Devi et al., 2019). In the present

study, the titratable acidity of the analysed lines exhibited variation (Figure 6), ranging from 1.40% (L10) to 2.40% (L7).

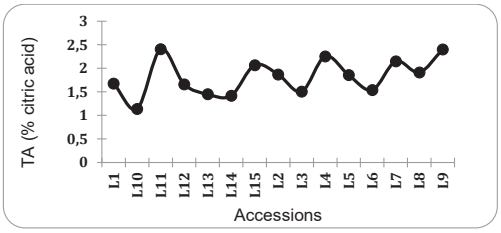


Figure 6. The mean values of TA content of pea accessions

According to Wu et al. (2023), the total dry matter content of peas typically ranges between 20% and 30%, depending on the pea variety and cultivation conditions. In our study, the recorded values fluctuated between 21.87% (L4) and 38.33% (L1) (Figure 7).

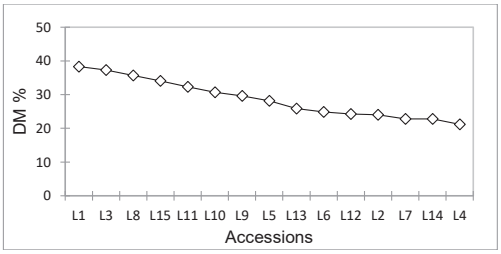


Figure 7. The mean values of DM content of pea accessions

***Phaseolus vulgaris* L.**

Soluble compounds, particularly sugars and organic acids, are fundamental to the energetic value and sensory properties of beans. A high total TSS content can be associated with an increased energy value, which may be relevant for the formulation of food products tailored to specific dietary requirements or nutritional needs (Bai et al., 2024).

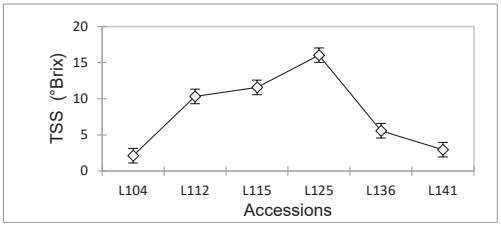


Figure 8. The mean values of TSS content of climbing bean accessions

In this study, TSS values ranged from 2.12°Brix (L104) to 16.03°Brix (L135) for climbing (Figure 8) beans and from 2.85°Brix (L4) to 13.23°Brix (L34) for dwarf beans (Figure 9).

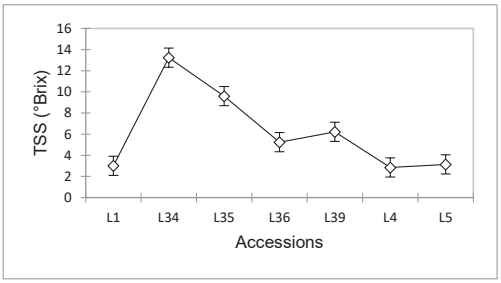


Figure 9. The mean values of TSS content of dwarf bean accessions

The acidity of beans represents a critical parameter in assessing both the quality and safety of bean-derived products, particularly in the context of processing and preservation. Acidity plays a pivotal role in shaping the sensory profile of the product while also influencing its resistance to microbial proliferation, including bacterial and fungal contaminants.

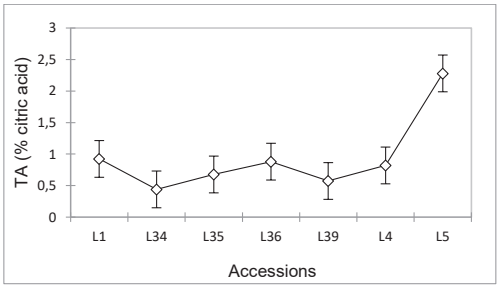


Figure 10. The mean values of TA content of dwarf bean accessions

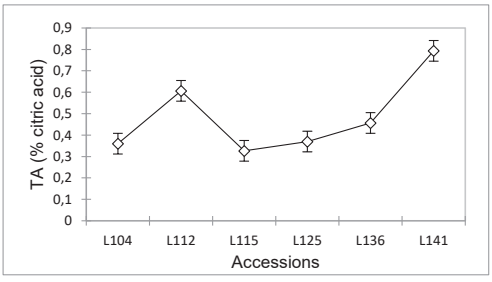


Figure 11. The mean values of TA content of climbing bean accessions

In general, beans are characterized by relatively low acidity levels (Gil et al., 2021), a trend corroborated by the findings of this study (Figure 10 and Figure 11). Specifically, the TA of climbing bean accessions ranged from 0.32 g/L (L115) to 0.79 g/L (L141), whereas dwarf bean accessions exhibited values ranging between 0.4 g/L and 2.20 g/L.

Total dry matter is another key indicator in the assessment of bean quality, as it determines both its nutritional value and its potential for various industrial applications, including the production of flour, protein concentrates, and other food products (Siddiq & Uebersax, 2022). The studied accessions exhibited total dry matter content ranging from 5.44% (L115) to 30.63% (L141) for climbing bean (Figure 12) and from 6.53% (L5) to 19.7% (L34) for dwarf beans (Figure 13). Overall, our findings indicate that climbing bean accessions, generally have a higher dry matter content than dwarf bean accessions, making them more suitable for flour production.

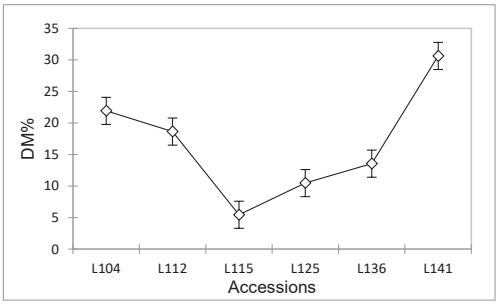


Figure 12. The mean values of DM content of climbing bean accessions

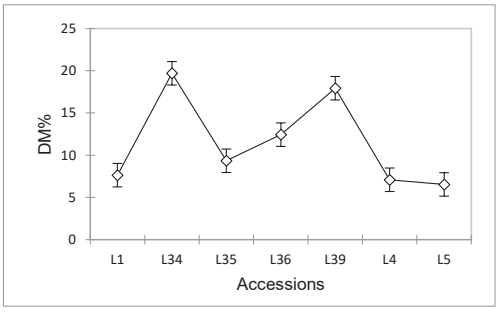


Figure 13. The mean values of DM content of dwarf bean accessions

CONCLUSIONS

This study highlights the significant variability in nutritional quality among legume species and genotypes. Broad beans (L5, L7) showed exceptional dry matter content, making them ideal for industrial applications. Peas (L6, L13) exhibited superior TSS values, suitable for fresh consumption, while climbing beans (L135, L141) showed high TSS, enhancing their potential for processing and storage.

These findings underscore the importance of exploiting genetic diversity to meet nutritional and processing needs. Future research should focus on optimizing environmental conditions and genotype selection to enhance the adaptability and nutritional profile of legumes under stress conditions such as drought or extreme temperatures.

ACKNOWLEDGEMENTS

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FRUIT QUALITY AND BIOACTIVE COMPOUND ACCUMULATION IN ROMANIAN PEPPER CULTIVARS

Ovidia Loredana AGAPIE, Elena BARCANU TUDOR, Ion GHERASE, Eliza TEODORESCU

Vegetable Research and Development Station Buzău, 23rd Mesteacănului Street, 120024, Buzău, Romania

Corresponding author email: ion2196@yahoo.com

Abstract

This study, conducted in 2023 and 2024, explored the phenotypic diversity of five Romanian pepper cultivars from VRDS Buzău (Roial, Decebal, Cantemir, Regal, L-50), focusing on fruit quality traits and yield. Fruit quality was assessed by analyzing dry matter content, fruit firmness, total soluble solids, titratable acidity, ascorbic acid, lycopene, and β-carotene at different ripening stages. Results showed a significant increase in bioactive compounds at full ripeness. L-50 exhibited the highest total soluble solids (9.53°Brix) and β-carotene (8.71 mg·100 g^{-1} F.W.) levels, making it nutritionally valuable. Roial stood out for its high vitamin C content (232.8 mg·100 g^{-1} F.W.), while Cantemir demonstrated superior firmness (11.42 N), enhancing post-harvest durability. All genotypes exhibited antioxidant properties, suggesting their potential contribution to functional foods These findings highlight the diversity and nutritional attributes of the studied Romanian pepper cultivars, though further research is needed to confirm their broader applicability.

Key words*: Capsicum spp., carotenoids, nutritional profile, vitamin C.*

INTRODUCTION

Capsicum peppers, part of the Solanaceae family, include 44 identified species, of which five are domesticated: *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens* (Barbosa et al., 2002; Agapie et al., 2024). Among them, *C. annuum* is the most widely cultivated and consumed in Romania due to its distinctive taste and pungency.

Globally, peppers are valued for their culinary versatility and rich nutritional profile, containing bioactive compounds with strong antioxidant properties (Bogusz et al., 2018).

Their consumption offers health benefits, including reduced risks of cardiovascular disease, diabetes, and neurodegenerative disorders. Additionally, they support brain function by preventing lipid oxidation in brain cells (Arimbor et al., 2015).

In 2021, global Capsicum production reached 38 million tons fresh and 5,8 million tons dry (FAO, 2023). In Romania, peppers rank fourth among vegetables, with 9,510 hectares cultivated, yielding 104,560 tons (FAO, 2023).

The Vegetable Research and Development Station (VRDS) Buzău plays a key role in breeding and preserving Capsicum germplasm, registering 11 pepper cultivars in the Official Catalogue of Species and Varieties of Cultivated Crops in Romania, including bell peppers, long peppers, pimiento peppers, and hot peppers.

In recent years, plant breeding efforts have increasingly focused on developing genotypes with high antioxidant content, recognizing the strong correlation between diet and human health (Brezeanu et al., 2022).

This study investigates the phenotypic diversity of five Romanian pepper cultivars, assessing key fruit quality traits and yield performance. The findings aim to highlight their potential applications while raising consumer awareness of their nutritional and health benefits.

MATERIALS AND METHODS

Five pepper varieties bred at VRDS Buzau were analyzed: two sweet peppers: Cantemir, Regal, two hot peppers: Roial, Decebal, and one pimiento accession L50 (Figure 1).

The experiment was conducted in the years 2023 and 2024 at the experimental research plot of the VRDS Buzău.

The crop technology applied for pepper cultivation followed the recommendations from the specialized literature (Barcanu et al., 2021).

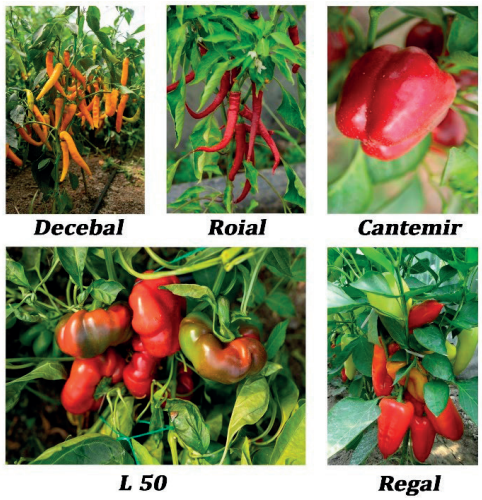


Figure 1. Pepper fruit varieties taken into study

Pepper fruits were harvested at two distinct ripening stages to assess fruit quality traits: harvest maturity (HM) and physiological maturity (PM). Fruits collected at the green stage (HM) were noted as Cantemir I, Regal I, Roial I, Decebal I, and L50 I, while fully ripe fruits at physiological maturity (PM) were noted as Cantemir M, Regal M, Roial M, Decebal M, and L50 M.

For fruit quality analysis, randomly mixed samples were prepared for each genotype, with all parameters assessed in triplicate on the harvest day. These included Total Dry Matter (%, AOAC 930.04), Total Soluble Solids (TSS - °Brix, AOAC 932.12), and Titratable Acidity (TA% citric acid, AOAC 942.15), using standardized methods.

The fruit firmness was tested using the electronic penetrometer TR Turoni 53205 equipped with a 3 mm piston and the results were expressed in Newton.

The quantification of lycopene and β-carotene was conducted according to the method developed by Nagata and Yamashita (1992) and the vitamin C content was determined using a modified iodine titration method (0.01 M) based on Sheeba and Shalini (2021). For juice preparation, 10 g of each sample was finely chopped, blended with 50 ml of distilled water, and filtered. The filtrate was transferred to a 100 ml volumetric flask and diluted to volume. A 20 ml aliquot was mixed with 150 ml of distilled water in a 200 ml Erlenmeyer flask, then titrated against iodine solution using an automatic TitroLine Easy. Results were expressed as mg/100 g fresh weight (FW).

Statistical analyses were performed using analysis of variance (ANOVA). In the case of a significant F-value, the means were compared using Duncan test.

RESULTS AND DISCUSSIONS

Hot peppers generally weigh less than sweet peppers. In this study, yield/plant ranged from 2.15 kg in Roial to 3.35 kg in L50 (Figure 2).

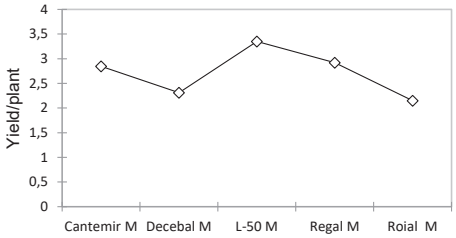


Figure 2. The mean values of yield/plant for studied varieties

Dry matter content (DM%) at harvest maturity ranged from 5.25% (Decebal) to 9.21% (Roial), increasing significantly during ripening, with Roial reaching the highest value (14.72%) and Regal the lowest (4.87%) (Figure 3). This trait is essential for chili powder production (Ribes-Moya et al., 2018).

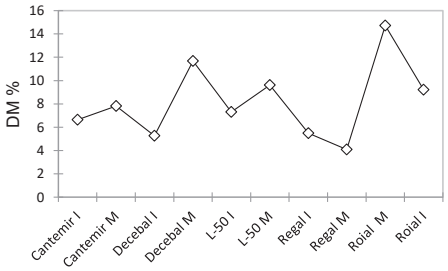


Figure 3. The mean values of DM content of studied varieties

Previous studies reported DM values between 4.83%-17.78% (Brezeanu et al., 2022) and 11.9%-16.5% (Stan et al., 2021), with the present study's range (4.87%-14.08%) falling within these intervals.

Cell wall structure is a key quality attribute influencing consumer preference and a primary selection criterion in breeding programs (Perez-Grajales et al., 2019). In this study, fruit firmness increased during ripening, peaking at 11.42 N in Cantemir at physiological maturity (Figure 4). High firmness enhances suitability for fresh consumption and extends shelf life (Barcanu et al., 2021), which is crucial for long-distance transport (Flores-Velázquez et al., 2022). Firmness values reported for bell peppers vary, ranging from 12.64N-21.60N (Figueroa et al., 2015) to lower values of 6.39N-7.0N (Palacio & Sánchez, 2015).

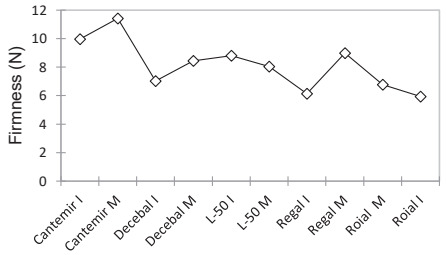


Figure 4. The mean values of firmness of studied varieties

TSS, comprising sugars and organic acids, is a key indicator of pepper maturity and sensory quality (Martínez et al., 2007). Similar to DM content, TSS increased during ripening, (Rahman et al., 2017). This rise enhances fruit sweetness due to hexose sugar accumulation (Nielsen et al., 1991).

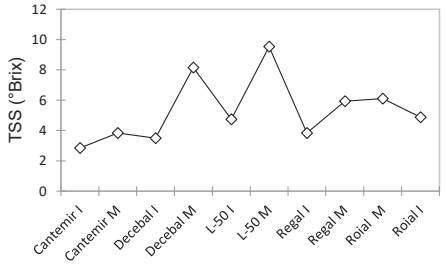


Figure 5. The mean values of TSS content of studied varieties

Our results (Figure 5) shown that TSS values varied across maturity stages but showed no statistical differences among genotypes. The highest TSS at harvest maturity was recorded in Roial, with 4.88°Brix, while at physiological maturity L50 shown the highest value, 9.33°Brix The recorded values (3.49-9.33°Brix) align with literature reports of 3.5-7°Brix (Soare et al., 2017) and 5.20-8.85°Brix (Flores-Velázquez et al., 2022).

Titratable acidity (TA) and the TSS/TA ratio serve as critical indicators of fruit ripening, reflecting the concentration of organic acids (Martínez et al., 2007). In this study, TA exhibited a significant increase during ripening (Figure 6), a trend consistent with the findings of Ghasemnezhad et al. (2011). At harvest maturity, the highest TA value was recorded in the Roial cultivar (0.18 g citric acid·100 g^{-1} FW), while the lowest was observed in the Decebal cultivar (0.09 g citric acid·100 g^{-1} FW). Notably, at physiological maturity, the Decebal variety demonstrated the most substantial increase in TA, reaching almost he highest content (0.27 g citric acid·100 g^{-1} FW).

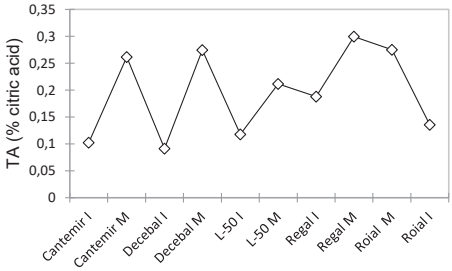


Figure 6.The mean values of TA content of studied varieties

The increasing interest in carotenoids stems from their potential health benefits, as peppers serve as a valuable source of lycopene and β-carotene (Brezeanu et al., 2022; Igbokwe et al., 2013). Lycopene has been associated with antioxidant, anti-inflammatory, and chemoprotective effects in cardiovascular and neurodegenerative diseases, as well as certain cancers (Cruz et al., 2013). Meanwhile, β-carotene, a precursor of vitamin A, exhibits strong free radical scavenging properties (Deepa et al., 2006).

Statistical analysis revealed significant differences in lycopene and β-carotene content among the studied cultivars. The highest lycopene concentration was recorded in the L50 variety (6.17 mg·100 g^{-1} F.W) (Figure 7), also

L50 accession exhibited the highest β-carotene content (8.71 mg·100 g^{-1} F.W) (Figure 8). In contrast, the Decebal cultivar, characterized by orange-colored fruits, had undetectable lycopene levels. Lycopene and β-carotene content in pepper (*Capsicum annuum*) genotypes is strongly influenced by genetic factors, with yellow and orange cultivars typically lacking lycopene due to mutations affecting carotenoid biosynthesis (Agarwal & Rao, 2000). These colour variations are primarily linked to differential gene expression regulating the conversion of β-carotene to downstream carotenoids, such as capsanthin and capsorubin, which are characteristic of red peppers (Rodríguez-Burruezo et al., 2011; Naresh et al., 2020).

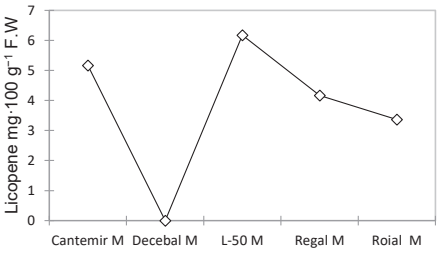


Figure 7. The mean values of licopene content of studied varieties

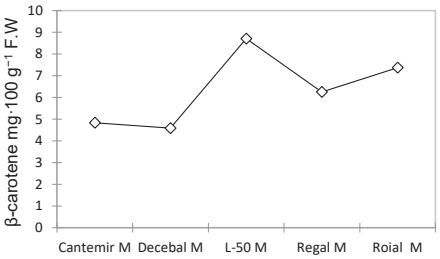


Figure 8.The mean values of β-carotene content of studied varieties

Carotenoid accumulation is closely linked to fruit ripening, as lycopene levels increase while chlorophyll degrades, leading to the transition from green to red pigmentation (Flores-Velázquez et al., 2022). Consequently, we analyze lycopene and β-carotene only at physiological maturity.

Peppers contain bioactive compounds with applications in animal feed, nutraceuticals, cosmeceuticals, and functional foods (Razola-Diaz et al., 2022). Epidemiological studies have consistently linked fruit and vegetable consumption to reduced mortality rates from cancer, cardiovascular diseases, degenerative disorders, and aging-related conditions (Chávez-Mendoza et al., 2015).

Vitamin C is among the most significant bioactive compounds in peppers, playing a crucial role in collagen biosynthesis and acting as a co-factor in essential hydroxylation reactions. Additionally, ascorbate supplementation has been associated with a reduced risk of Alzheimer's disease, while its antioxidant properties help mitigate cellular damage (Grosso et al., 2013). Since humans cannot synthesize vitamin C due to mutations in the gluconolactone oxidase gene, dietary sources of this nutrient are essential.

In this study, vitamin C content varied significantly among genotypes and increased during ripening (Figure 9). At physiological maturity, Roial exhibited the highest concentration (232.80 mg/100 g FW), while Regal and L50 showed the lowest levels (94.93-106.73 mg·100 g^{-1} F.W). Considerable variation in ascorbic acid content has been reported in previous studies (Dinu et al., 2013; Soare et al., 2017), influenced by cultivar response, sampling variability, and environmental factors (Kouassi et al., 2012).

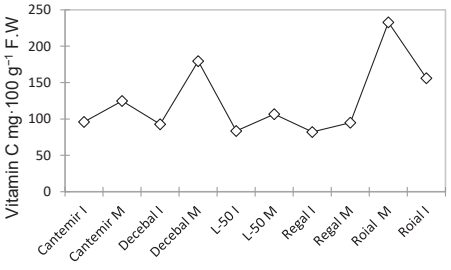


Figure 9.The mean values of Vitamin C content of studied varieties

All analyzed pepper varieties proved to be excellent sources of vitamin C, surpassing the recommended daily dose (RDD) of 60 mg/ 100 g. On average, Regal contributed 158% of the RDD, while Roial provided 348%. These findings align with previous studies suggesting that bell peppers (*Capsicum annuum*) can

provide between 25% and 218% of the recommended daily dietary intake (RDD) of vitamin C, depending on factors such as genotype, ripening stage, and cultivation conditions (Chávez-Mendoza et al., 2015).

CONCLUSIONS

The study provides insights into the physical attributes, bioactive compound composition, and yield potential of five Romanian pepper (*Capsicum annuum*) cultivars. Among the evaluated genotypes, L-50 exhibited the highest total soluble solids and β-carotene content, suggesting its potential nutritional value. Roial demonstrated elevated vitamin C levels, while Cantemir displayed notable fruit firmness, which may contribute to improved postharvest storage and transportability.

These findings contribute to a broader understanding of the phytochemical profile of these pepper cultivars. The studied varieties show promising characteristics for direct market use, though additional investigations on consumer preference and postharvest behaviour would support their commercial recommendations.

ACKNOWLEDGEMENTS

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EFFECT OF CULTIVAR AND GROWING SYSTEM ON THE PHENOLOGICAL AND QUALITY ATTRIBUTES OF TOMATO

Andreea ANTAL-TREMURICI[1], Creola BREZEANU[1], Alexandru BUTE[1], Claudia BĂLĂIȚĂ[1], Mariana CALARA[1], Vasile STOLERU[2]

[1]Vegetable Research and Development Station Bacău, 220 Bârlad Street, Bacău, Romania
[2]"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: antal_andreea97@yahoo.com

Abstract

Tomato is a staple crop with diverse cultivars, and their adaptability to environmental changes prevalently derives from distinct genetic traits that influence their phenology, fruit quality, and antioxidant constituents. Also, environmental conditions and agronomic techniques have an effect on these. This experiment was carried out at the VRDS Bacau in subdivided plots, with three replicates in protected areas in which the influence of two factors was evaluated: the cultivar (genotype 1 to genotype 10) and the growing system (conventional and organic). Data collection involved phenological observations (flowering time, fruit set, maturation, and overall growth) and chemical analyses (acidity, carotenoid content). Regarding the influence of the cultivar on the phenological characteristic values are reached by G2 for soluble solids, G4 for TSS, G1 for water%, and G2 for lycopene and beta carotene. Tomatoes grown organically were found to have richer flavour profiles and deeper colour intensity compared to their conventionally grown counterparts, which tended to be more uniform in size and shape.

Key words*: genotypes, growing system, plant breeding, phenological properties, nutritional quality.*

INTRODUCTION

Tomatoes are among the most widely cultivated and consumed vegetable crops worldwide, playing a vital role in human nutrition and culinary traditions. As a member of the *Solanaceae* family, tomatoes showcase an impressive range of morphological, flavor, color, and growth trait diversity. This variation can be traced back to their long history of domestication and selective breeding. Understanding the classification of tomato cultivars is essential for comprehending their agricultural, genetic, and culinary characteristics. A cultivar, short for "cultivated variety", is a plant variety developed through selective breeding, distinguishing it from wild species. Tomato cultivars can be classified based on several key criteria, including growth habit (determinate vs. indeterminate), fruit type (e.g., cherry, beefsteak, plum), and resistance traits (such as resistance to specific pests or diseases). Additionally, cultivars can be further categorized as heirloom or hybrid varieties, each offering unique traits that cater to specific farming practices and consumer preferences. This classification system helps to shed light on the diverse genetic makeup and breeding potential of tomatoes, which is crucial for ensuring agricultural productivity and sustainability. The selection of tomato cultivars is influenced by various factors, such as ease of germination, cold tolerance, uniformity, absence of imperfections, reduced axillary buds, and resistance to diseases and pests (Umeohia and Olapade, 2024).

The phenological development of plants determines their growth and productivity. Days to blooming, fruiting, and crop maturity are significant phenological events that impact a crop's productivity. In 2023, Donoso and Salazar, in a study about plant agromorphology of indeterminate tomato, demonstrated that the landraces studied showed great diversity of agromorphological and phenological traits, and this diversity might be key to yield improvement in tomatoes.

The fruit's maturity, cultivar, soil, growing system, temperature conditions, and the farming technique used to develop the plants all affect the tomato fruit's chemical composition (Coyago-Cruz et al., 2018; Hasnain et al., 2020).

The primary components that contribute to the quality of tomatoes are glucose, fructose, citric acid, lycopene, β-carotenoids, quercetin, chlorogenic acid, and succinic acid (Li et al., 2024). The lycopene and antioxidant levels in tomato genotypes vary among cultivars (Taber et al., 2008). Tilahun *et al*. (2017) observed that the cultivar had a significant effect on the phenolic content of tomato fruits. Ilić et al. (2013) confirm that the most important variable in the micronutrient content of tomatoes is cultivar. According to Martínez-Valverde et al. (2002), the concentrations of lycopene and the various phenolic compounds (flavonoids (quercetin, kaempferol, and naringenin) and hydroxycinnamic acids (caffeic, chlorogenic, ferulic, and p-coumaric acids), as well as the antioxidant activity, were significantly influenced by the tomato variety. According to research by Cortés-Olmos et al. (2014) and Kavitha et al. (2014), tomato cultivars, especially traditional varieties, vary widely in terms of their carotenoid levels, which leads to a significant source of genetic variation. Also, Flores et al. (2017) found substantial variation throughout 53 traditional tomato cultivars in terms of size, shape, color, carotenoid profiles, and concentrations. A study undertaken to analyze the genotypic variability linked with certain traits in twenty-five distinct tomato genotypes revealed that the evaluated genotypes possessed significantly significant variation (Zannat et al., 2023).

Typically, conventional farming systems use synthetic pesticides, herbicides, and fertilizers to increase yields and manage diseases and pests. Because the inputs in these systems are frequently more controlled, yields may be higher, but there are also worries about the environmental impact and consumer preference for chemical-free products. On the other hand, organic farming methods place more emphasis on crop rotation, natural inputs, and biological pest control, all of which can have a different effect on fruit qualities, disease resistance, and plant growth than conventional methods. Although organic tomato production is thought to be healthier and more ecologically friendly, there is ongoing discussion regarding yield and quality issues. A key variable in the success of the organic tomato culture is variety selection. In fact, the variety's genetic components can affect a number of crucial aspects, including fruit quality, disease resistance, and production potential. Research results on the effects of organic and conventional production on fruit quality are sometimes contradictory. Several studies report better taste, higher vitamin C contents, and different levels of other quality-related compounds (Ruiz Espinoza et al., 2021; Vélez-Terreros et al., 2021), whereas several other studies have found the opposite or no differences in quality characteristics between organically and conventionally grown vegetables (Kapoulas et al., 2011). Organic cultivation provided an advantage to obtaining fruits and vegetables with higher amounts of phenolic compounds, which brings additional value to these crops in terms of antioxidant potency as well as a lower environmental impact (Basay et al., 2021). By keeping diseases and pests under control, organic agriculture maintains the natural balance, ensures soil fertility, and optimizes the use of energy and natural resources while preserving the continuity of existence in the environment (Yüzbaşioğlu, 2018). While some research has indicated that organically grown tomato fruits contain more bioactive compounds than conventionally produced ones, not all of these studies have been consistent. The amounts of phenolics and carotenoids vary substantially and are frequently influenced by cultivation, genotype, and ripeness. Hallmann (2012) demonstrated that the organic growing system affects tomatoes quality parameters, such as nutritional value and phenolic compound content, and also that the second significant factor of the nutritional value of tomatoes is the type of fruit. In general, the application of organic fertilizers significantly improved most of the qualities, such as TSS, SS, lycopene, and nitrate content, with no significant change in the sugar/acid ratio (Gao et al., 2023; Fan et al., 2023; Stoleru et al., 2024). Also, Borguini et al. (2013) confirmed that the type of production (conventional and organic) would affect the production of secondary metabolites by the plants. Mohammed et al. (2020) show that tomatoes fruits cultivated in an organic system were significantly different in mineral nutrients and dry matter content from a diverse genetic background point of view. Vélez-Terreros *et al*. (2024) did another study that demonstrated that

while organic tomatoes had a slightly better nutritional composition, including AA, lycopene, and total phenolics, the nutritional and nutraceutical properties of the final product may depend on the cultivar, harvest time, genetic and agricultural factors, growing conditions, and production region.

Measuring the growth and harvesting traits of the local tomato population, such as plant height, fruit set percentage, and overall yield, is essential for determining the viability of plastic tunnels for vegetable production. The analysis of both types' productive performance and qualitative features (such as fruit size, shape, color, and taste) is critical for selecting the best assortment and assuring production efficiency.

Hence, in the present work, a specific survey on tomato, within a protected field long-term study on organic and conventional horticulture and the different genotypes of tomato, is presented. Since the variables affecting tomato quality are complex and interconnected, more research is required to fully understand the interdependencies. The main objective of the present study was to assess how the combination of tomato genotypes and different growing systems impacts the phenology, morphology, and quality of the fruits, providing insights into the best practices for growing tomatoes with superior quality, efficiency, and productivity.

MATERIALS AND METHODS

The plant material consisted of 10 cultivated tomato genotypes belonging to diverse cultivar groups, including elite cultivars and heirlooms with different origins, landraces for the fresh market with determinate and indeterminate growth from Romania originating from different regions of this country. The panel set was assembled from the germplasm collections of the Vegetable Research and Development Station from Bacau, Romania. The research was carried out in the organic and conventional systems in plastic tunnels during spring and summer 2024 at VRDS Bacau, characterised by a typical temperate climate (46°5846201″ N, 26°9511173"E, 158.96 m a.s.l.).

The experiment was set up using seedlings produced in alveolar pallets with 70 alveoli, and sowing was carried out on March 5th using a textured substrate and a medium fertilisation comprising microelements. Manual planting was performed after the seedling had become fully developed in the third decade of April, at 48 days old. During the vegetation period, frequent assessments on pest and disease resistance, as well as plant development progress in protected areas, were performed. Manual weeding was done around the plants whenever necessary and at frequent times, and Mospilan treatments were used in the conventional method to combat pests and diseases. Cropmax and Codamix were used to fertilise the plants in the organic system and Complex 20:20:20 in the conventional one.

The experiment was divided into 5 furrows with two rows in each furrow, a one-meter path between each variety, 35 cm between plants, and 70 cm between rows. Each variant had 10 plants, and the space required for each plant was 0.245 sq m.

During the vegetation period, measurements of the crown diameter and plant height were made, the date of flowering, the appearance of the first fruit, and the ripening of the fruit were noted to subsequently calculate the specific number of days for each parameter. In our experiment, 7 measurements of height (first measurement before planting, then two weeks apart) and diameter at the base of tomato plants were made, each being scored from 1 to 7 for each parameter. HP1 - height in a certain phenophase, DP - stem diameter in a certain phenophase (HP1 ... Hp7; DP1 ... DP7). To measure the diameter of plant base and fruit diameter and the fruit height, a caliper was used that shows values in both inches and mm, and to measure the fruit mass, a Kern analytical balance was used.

The dry matter (DM) content was determined by drying the freshly collected samples for 24 hours at 70 ± 2°C in a forced air-drying oven (Biobase) to provide a uniform mass (Stoleru et al., 2020; Caruso et al., 2019). Tomato juice was squeezed from fresh tomatoes onto a digital refractometer to identify total soluble solids (TSS), and outcomes were expressed in °Brix using AOAC method 932.12 (AOAC, 2005).

The pigments, lycopene and β-carotene, were assessed using spectrophotometry. The absorbance of the etheric extract was measured at several wavelengths, 452 nm for carotene and 472 nm for lycopene, using a BOECO S-20 spectrophotometer in comparison to a petroleum

ether blank. The absorbance values for lycopene and β-carotene were multiplied with 14.105, respectively, with 19.96 to calculate the total fractions.

For statistical analysis, the IBM SPSS Statistics application, version 26.0, was utilized. Tukey's test was used to estimate the significant difference between the variant means. Differences across groups were considered significant at $p \leq 0.05$. The results were given as means and standard deviations.

RESULTS AND DISCUSSIONS

Tallness, shortness and other morphological differences are varietal characteristics, which are controlled and expressed by certain genes (Fayaz et al., 2007). Plant height increased gradually with time and continued up to 100% flowering (Biswas et al., 2014; Sanjida et al., 2020). Plant height differed among the varieties of tomato due to the variation of varieties (Olaniyi et al., 2010). Amare and Gebremedhin (2020) found in an experiment that the tallest tomato plant has a height of 112 cm and the shortest has a height of 73 cm. In another study, it was discovered that the highest plant height was 93.8 cm in V2 (BARI hybrid tomato), and the lowest (87.3 cm) was found in V1 variety at 60 DAT (Sanja et al., 2020). According to Fayaz et al. (2007), the genetic potentiality of summer tomato varieties may be the cause of the variations in plant height.

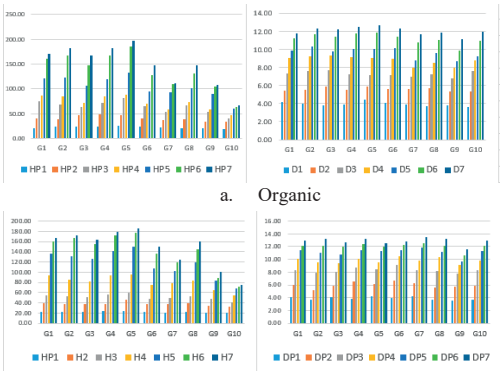


(G1 - genotype 1, …., G10 - genotype 10, HP - height in a certain phenophase; DP - stem diameter in a certain phenophase)

Figure 1 Plant height and diameter at the base of the plants depending on the cultivar

In a study conducted in Iasi in 2018-2019, Stoleru et al. (2020) demonstrated that the height of tomato plants depending on the cultivar showed values between 206.4 and 224.2 cm; however, depending on the cultivation system, the maximum was reached at 227 cm for the conventional system and 204.4 for the organic system.

As can be seen in the graph (Figure 1), the values increase progressively for each genotype from one time interval to another. From the point of view of cultivar analysis, G5 reached the maximum height value of 191.11 cm, while the lowest value was reached by G10 (70.67 cm). In the case of the diameter at the base, G4 (12.60 mm) reached the maximum point, and G9 (11.35 mm) the minimum value. From a statistical point of view, it is observed that there are no significant differences, demonstrating a natural growth process.



G1 - genotype 1, …., G10 - genotype 10, HP - height in a certain phenophase; DP - stem diameter in a certain phenophase)

Figure 2. Plant height and diameter at the base of the plants depending on the growing system

It can be seen that the growth trend is also upward in the case of the influence of the growing system, the maximum values being reached by G5, and the minimum values by G10 in both cases for the plant height. In the case of the diameter at the base of the plant, there are no significant differences that can be observed between the values of measurement no. 7. Following the analysis of these graphs, it can be concluded that the genotypes G1, G2, G3, G4, G5, G6, and G8 present an indeterminate growth of the plant, specific to protected spaces, solariums, being followed by G7, G9, and G10, which present a determinate growth.

The flowering stage of the tomato plant begins when the plants have burst into a yellow bloom that ends in small green fruits after pollination. The number of flowers initiated in an inflorescence depends on the variety and environmental conditions. Increased irradiance or decreased plant density and decreased temperatures positively influence the number of flowers formed in an inflorescence. Low air temperatures (<10°C) during inflorescence initiation promote inflorescence branching, usually resulting in more flowers per inflorescence (Wien and Stützel, 2020). Furthermore, Abdelmageed and Gruda (2009) and Singh et al. (2014) claimed that environmental and genotypic factors affect tomato plants' ability to flower early or late. According to some scientific works, the number of days until 50% flowering was between 40 and 49 days (Meseret and Kassahun, 2012), so 49.67 and 67 days conform to Tujuba and Ayana, 2020. Their inherited traits, which include early acclimatization to the growing environment to promote growth and development, may have contributed to their early or late flowering days. In our study, depending on the cultivar, the data show significant variations between the values of the genotypes studied, the data being between 63.83 (G9) and 75.83 (G2) days from emergence. Taking into account the day the genotypes were planted, the number of days until the appearance of the first flower is between 16 and 28 days.

Regarding the crop system, the values demonstrate significant differences between the cultivars, in the conventional one, the genotype that bloomed the fastest being G9 with 54.33 days from emergence, and the most delayed genotype being G2 with 71.67 days. In the organic crop system, the values varied between 71.00 (G10) and 80 days (G2).

Table 1. Phenological data depending on the cultivar

V	Days till starting flowering	Days till fruit development	Days till harvest
G1	73.17±1.17 ab	81.00±0.67 ab	111.33±0.59 bc
G2	75.83±1.06 a	84.50±0.46 a	118.83±0.09 a
G3	69.67±1.57 bc	77.50±1.33 bc	112.50±0.57 bc
G4	72.00±1.58 ab	81.83±1.04 ab	116.83±0.57 a
G5	72.33±1.46 ab	81.50±1.26 ab	113.50±1.27 b
G6	70.67±1.40 bc	78.83±0.96 bc	113.17±0.35 bc
G7	67.00±1.96 cd	74.50±1.42 cd	111.83±1.08 bc
G8	69.50±1.43 ab	79.67±1.28 ab	112.17±1.18 bc
G9	63.83±2.37 d	71.83±2.14 d	109.50±1.33 c
G10	65.833±1.46 bc	76.67±0.57bc	110.50±0.63 bc
Ss.	*	*	*

(V – variant; G1 – genotype 1, …., G10 – genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

The number of days until tomatoes bear fruit depends on several factors, including variety, as different tomato varieties have different ripening times. In a work by Shopova, 2023, in Bulgaria, a delay in the fruiting phenophase of plants is demonstrated because after the 2nd decade of July, flowers are more likely to fall under higher temperature conditions. Thus, in the first year of the experiment, 2020, the first fruit appeared at 47, 48, and, respectively, 52 days, while in the second year of the experiment, 2021, the first fruit appeared slightly later at 48, 49, and 54 days after planting. The number of days until the appearance of the first fruit was significantly influenced by the cultivar produced. The shortest time was obtained by the genotype G9 with 71.83 days from emergence, while the maximum number of days was represented by G2. By interpreting the results, several genotypes are statistically similar in pairs, such as G1 (81.00) with G4 (81.83), G5 (81.50), and G8 (79.67), but also G3 (77.50) with G6 (78.83). When we refer to the crop system, in the organic one the values are statistically significant, the lowest results being obtained by G10 with 78.83 days and the highest being represented by G5 with 86.67 days from emergence. In the conventional system, the genotype that produced the earliest fruit is G9 (63.33), a genotype with determinate growth, and the longest time is given to the G2 genotype.

Table 2. Phenological data depending on the growing system*: a. organic*

V	Days till starting flowering	Days till fruit development	Days till harvest
G1	77.67±0.60 abc	83.67±0.17 bc	112.33±0.17 d
G2	80.00±0.29 a	86.00±0.29 ab	119.00±0.00 a
G3	76.0±0.29 bcde	82.67±0.60 cd	114.33±0.44 cd
G4	78.33±0.44 ab	85.67±0.60 ab	118.33±0.83 ab
G5	78.33±0.17 ab	86.67±0.17 a	118.00±0.00 ab
G6	76.33±0.17 bcd	82.33±0.60 cd	113.00±0.00 d
G7	74.67±0.44 de	79.67±0.73 e	115.33±1.36 bcd
G8	75.00±0.29 cde	84.67±0.17 abc	116.67±0.88 abc
G9	73.33±1.01 ef	80.33±1.20 de	114.33±1.17 cd
G10	71.00±1.50 f	78.33±0.67 e	112.67±0.60 d
Sf.	*	*	*

b. conventional

V	Days till starting flowering	Days till fruit development	Days till harvest
G1	68.67±0.60 b	78.33±0.33 b	110.33±1.09 cd
G2	71.67±0.60 a	83.00±0.50 a	118.67±0.17 a
G3	63.33±0.60 d	72.33±0.67 d	110.67±0.60 c
G4	65.67±0.60 cd	78.00±0.76 b	115.33±0.33 b
G5	66.33±0.17 bc	76.33±0.17 bc	109.00±1.32 cd
G6	65.00±0.58 cd	75.33±0.73 c	113.33±0.73 b
G7	59.33±1.20 e	69.33±1.17 e	108.33±0.17 cd
G8	64.00±1.04 cd	74.67±0.83 cd	107.67±0.33 d
G9	54.33±0.60 f	63.33±0.17 f	104.67±0.60 e
G10	60.67±0.44 e	75.00±0.50 c	108.33±0.44 cd
Ss.	*	*	*

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

Tomatoes can be grown in both temperate and tropical areas. Tomato ripening is accompanied by changes in the color of the tomato fruit, which acquires shades of red, pink, yellow, orange, or even black, typical of numerous hybrids. The color change signals the production of specific acids and sugars that build the flavor of tomato fruits. Different researchers (Chernet et al., 2013; Dufera, 2013) reported a wide range of differences in maturity (73 - 93 days and 69 - 156 days) for 21 tomato genotypes and, respectively, for 36 tomato genotypes. Fayaz Ahmad et al. (2007) stated that early or late maturity is attributed to genotypic character and to the extent that it is influenced by environmental factors of a particular growing area. The results of our study regarding the cultivar show that the maximum number of days to reach fruit maturity was 118.83 days (G2), an indeterminate genotype being considered in our case among the latest genotypes. The minimum number of days is reached by G9 with 109.50 days from emergence, being considered to be the most typical. Mitul et al. (2016) demonstrated in the study of 14 tomato genotypes that the number of days until first flowering varies between 74.64 and 82.50 days, while the number of days until first ripening is between 128.60 and 135.10 days.

The Tabel 2 a and b show us how the crop system influences the number of days to fruit ripening. Therefore, in the conventional system, the G9 genotype obtains the lowest number, namely 104.67 days, and the highest is represented by G2 (118.67). When we refer to the organic system, the values for G1 (112.33), G6 (113.00), and G10 (112.67) are considered statistically similar, these genotypes being considered the earliest and most suitable for this type of system. The latest genotype is represented by G2 (119.00).

Morphological characterization describes plant species using expressed phenotypes (Table 3). This method identifies key agronomic traits that can help with breeding, crop production, and the preservation and use of germplasm. Also, morphological traits are important diagnostic features that can be used for distinguishing genotypes (Chime et al., 2017).

In our study, depending on the cultivar, the lowest fruit length (5.64 cm) was found in genotype G7, while the highest value (8.57 cm) was measured in the landrace 4. Glogovac et al. (2022) reported a variation in fruit length from 3.8 to 6.3 cm in twenty tomato genotypes.

Table 3. Morphological data depending on the cultivar

V	Fruit diameter	Fruit length	Fruit mass
G1	88.57±2.33 b	61.57±1.30 e	281.50±18.42 b
G2	79.71±1.40 c	66.38±0.89 d	238.10±10.64 c
G3	71.30±1.42 d	56.75±0.71 f	168.55±8.86 d
G4	86.95±2.15 b	85.75±1.50 a	308.22±14.32 b
G5	98.60±2.04 a	75.00±1.09 b	348.50±16.73 a
G6	73.07±1.15 d	60.79±0.64 e	178.08±6.02 d
G7	61.06±0.85 e	56.43±0.56 f	122.36±4.19 ef
G8	68.66±1.29 d	70.62±1.17 c	168.64±7.90 d
G9	67.48±0.90 d	59.24±0.64 ef	145.69±4.84 de
G10	50.50±0.48 f	60.74±0.61 e	84.77±1.85 f
Ss.	*	*	*

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

Referring to the crop system, the values are statistically significant, which proves that the way tomato plants are cultivated significantly influences the final results.

Table 4. Morphological data depending on the growing system: *a. organic*

V	Fruit diameter	Fruit length	Fruit mass
G1	75.75±2.14 b	55.06±1.02 de	178.40±11.18 c
G2	78.45±1.79 b	65.18±1.23 c	229.67±14.08 b
G3	64.43±1.09 cd	54.73±0.74 de	131.32±6.36 cde
G4	79.13±2.54 b	83.13±2.05 a	250.46±17.29 b
G5	93.92±2.77 a	74.70±1.54 b	322.63±23.26 a
G6	68.56±1.58 c	59.01±0.84 de	157.49±8.36 cd
G7	58.04±1.11 d	54.50±0.74 e	106.76±5.17 ef
G8	64.87±1.73 cd	65.37±1.19 c	139.05±8.82 cde
G9	64.75±1.16 cd	57.13±0.94 de	128.38±4.89 de
G10	49.32±0.69 e	59.63±0.94 d	78.29±2.17 f
Ss.	*	*	*

b. conventional

V	Fruit diameter	Fruit length	Fruit mass
G1	101.40±2.48 ab	68.08±1.70 c	384.60±22.86 a
G2	80.96±2.15 c	67.58±1.28 c	246.53±16.05 b
G3	78.17±1.94 cd	58.76±1.12 d	205.78±13.56 bc
G4	94.77±2.84 b	88.37±2.12 a	365.98±17.46 a
G5	103.29±2.78 a	75.31±1.56 b	374.36±23.50 a
G6	77.58±1.23 cde	62.57±0.86 d	198.67±6.97 bc
G7	64.07±1.05 g	58.35±0.70 d	137.96±5.26 de
G8	72.45±1.66 ef	75.87±1.51 b	198.22±10.74 bc
G9	70.21±1.19 fg	61.36±0.69 d	163.01±7.13 cd
G10	51.67±0.62 h	61.85±0.73 d	91.24±2.51 e
Ss.	*	*	*

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

In the conventional system (Table 4 b), the G4 genotype obtained the highest value for both fruit length (88.37 mm) and fruit weight (365.98 g). The latter value showed a statistically significant correlation with the G5 genotype (374.36 g). The lowest fruit length was recorded by G7 (58.35 mm), with values statistically similar to those obtained by G3, G6, G9, and G10. The minimum fruit weight was found in G10, with a value of 91.24 g.

Regarding fruit diameter, G5 exhibited the maximum value (103.29 mm), while G10 had the smallest (51.67 mm) (Table 4 b). Stoleru et al. (2020) reported fruit mass values ranging from 161 to 183 g, depending on the cultivar and cultivation system. In the organic system, the average fruit mass was 175 g, while in the conventional system, it was 160 g.

As a result of analysing the key indicators of the biochemical composition of tomato fruits, significant differences were observed among the studied accessions. One of the key indicators of tomato fruit quality and its technological properties is the dry matter (DM) content. In an experiment carried out in 2021, Kurina et al., 2021 demonstrated that the dry matter content in the fruits of cultivated tomatoes was in the range of 3.72-8.88% (Cv = 14.7%), and in the fruits of wild species - 9.62- 11.33% (Cv = 6.2%). Fruits with a high concentration of dry matter not only taste better but also yield more during processing, and offer superior transportability and longer shelf life during storage. In our study, the dry matter content did not show statistically significant differences across cultivars. The genotype values ranged from 4.24 to 5.39, with the highest value recorded for G4 and the lowest for G1. Regarding the cropping systems, the conventional system yielded similar results to the organic system, with values ranging from 3.87 to 5.82 in the conventional system, and from 4.19 to 6.18 in the organic system. These results suggest that the organic cropping system positively influences dry matter accumulation, as indicated by the higher values observed.TSS content has a significant impact on fruit flavor and increases yield while lowering dehydration costs during processing, making it a crucial indicator of technological quality. From a cultivar point of view, TSS varied from 4.55 in genotype G9, which is statistically similar to G10 (4.63) (Table 5), to 6.81°Brix in the traditional variety G2. Some researchers reported similar TSS variation from 2.02 to 4.57°Brix (Aoun et al., 2013), 3.4 to 6.8°Brix (Henareh et al., 2015), and 4.6 to 6.3°Brix (Glogovac et al., 2022).

Depending on the cultivation system, the genotype with the highest total soluble solids value in the conventional system was G2 (6.74), while the lowest value was observed in G9 (4.35), which was statistically similar to G10 (4.40) (Table 6 b). In a study of 12 tomato genotypes, Mahmoud and Osman, 2023 showed that total soluble solids ranged between 3.87 °Brix and 5.57. In the organic system, significant differences were observed, with values ranging from 4.75 (G9), statistically similar to G10 (4.87), to 7.00 (G5), which was statistically comparable to G2 (6.87) (Table 6 a). Swetha et al. (2018) demonstrate that the organic tomatoes have a 4.7 °Brix, while those inorganics have 4.1°Brix. Also, similar results (4.76 and 5.08°Brix) were obtained by de Zoran et al. (2014) in an organic system.

Table 5. Physiological characteristics depending on cultivar

V	TSS (%)	DM %	Water%	Lycopene mg 100 g $^{-1}$ F.W.	β-carotene mg 100 g $^{-1}$ F.W.
G1	5.78 ± 0.03 cd	4.24 ± 0.51	95.76 ± 0.51	8.37 ± 0.45	12.47 ± 0.91
G2	6.81 ± 0.04 a	5.02 ± 0.61	94.98 ± 0.61	9.09 ± 0.34	13.98 ± 0.67
G3	5.92 ± 0.03 bcd	4.87 ± 0.21	95.13 ± 0.21	8.17 ± 0.51	12.18 ± 0.92
G4	6.03 ± 0.06 bc	5.39 ± 0.47	94.61 ± 0.47	8.54 ± 0.35	12.80 ± 0.78
G5	6.40 ± 0.28 ab	4.30 ± 0.35	95.70 ± 0.35	8.38 ± 0.38	12.62 ± 0.71
G6	5.45 ± 0.06 d	4.25 ± 0.46	95.75 ± 0.46	8.02 ± 0.33	11.92 ± 0.59
G7	5.47 ± 0.09 d	5.09 ± 0.45	94.92 ± 0.45	7.71 ± 0.76	11.55 ± 1.41
G8	5.52 ± 0.14 d	5.32 ± 0.31	94.68 ± 0.31	8.56 ± 0.54	12.89 ± 1.10
G9	4.55 ± 0.10 e	4.64 ± 0.42	95.36 ± 0.42	7.60 ± 0.74	11.35 ± 1.31
G10	4.63 ± 0.11 e	5.23 ± 0.34	94.77 ± 0.34	7.77 ± 0.58	11.60 ± 1.02
Sf.	*	ns	ns	Ns	ns

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

Table 6. Physiological characteristics depending on the growing system*: a. organic*

V	TSS (%)	DM %	Water%	Lycopene mg 100 g $^{-1}$ F.W.	β-carotene mg 100 g $^{-1}$ F.W.
G1	5.73 ± 0.02 bc	4.19 ± 0.97	95.81 ± 0.97	9.39 ± 0.03 bc	14.49 ± 0.06 b
G2	6.87 ± 0.06 a	6.18 ± 0.59	93.82 ± 0.59	9.85 ± 0.02 a	15.46 ± 0.04 a
G3	5.89 ± 0.06 bc	5.04±0.12	94.96 ± 0.12	9.31 ± 0.05 bc	14.24 ± 0.04 c
G4	6.00 ± 0.09 b	4.96 ± 0.66	95.04 ± 0.66	9.33 ± 0.03 bc	14.54 ± 0.04 b
G5	7.00 ± 0.18 a	4.31 ± 0.09	95.69 ± 0.09	9.23 ± 0.02 c	14.21 ± 0.06 c
G6	5.54 ± 0.03 cd	4.71 ± 0.51	95.29 ± 0.51	8.75 ± 0.07 e	13.24 ± 0.11 e
G7	5.30 ± 0.05 d	5.68 ± 0.02	94.32 ± 0.02	9.41 ± 0.03 b	14.71 ± 0.03 b
G8	5.82±0.04 bc	5.44 ± 0.04	94.56 ± 0.04	9.76 ± 0.01 a	15.35 ± 0.01 a
G9	4.75 ± 0.08 e	4.72 ± 0.56	95.28 ± 0.56	9.24 ± 0.00 c	14.27 ± 0.03 c
G10	4.87 ± 0.08 e	5.25 ± 0.75	94.75 ± 0.75	9.07 ± 0.03 d	13.88 ± 0.03 d
Ss.	*	ns	ns	*	*

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

b. conventional

V	TSS (%)	DM %	Water%	Lycopene mg 100 g $^{-1}$ F.W.	β-carotene mg 100 g $^{-1}$ F.W.
G1	5.82 ± 0.05 bc	4.29 ± 0.60	95.71 ± 0.60	7.36 ± 0.04 cd	10.4 ± 0.07 c
G2	6.74 ± 0.03 a	3.87 ± 0.41	96.13 ± 0.41	8.32 ± 0.02 a	12.49 ± 0.01 a
G3	5.95 ± 0.04 bc	4.69 ± 0.43	95.31 ± 0.43	7.03 ± 0.03 e	10.11 ± 0.03 d
G4	6.05 ± 0.09 b	5.82 ± 0.69	94.18 ± 0.69	7.76 ± 0.02 b	11.05 ± 0.10 b
G5	5.80 ± 0.12 bc	4.30 ± 0.77	95.70 ± 0.77	7.54 ± 0.02 c	11.03 ± 0.01 b
G6	5.36 ± 0.09 de	3.80 ± 0.77	96.20 ± 0.77	7.28 ± 0.01 d	10.60 ± 0.01 c
G7	5.63 ± 0.10 cd	4.49 ± 0.80	95.51 ± 0.80	6.02 ± 0.01 g	8.39 ± 0.01 f
G8	5.21 ± 0.07 e	5.20 ± 0.68	94.80 ± 0.68	7.36 ± 0.01 cd	10.43 ± 0.05 c
G9	4.35 ± 0.08 f	4.56 ± 0.74	95.44 ± 0.74	5.95 ± 0.12 g	8.43 ± 0.16 f
G10	4.40 ± 0.02 f	5.22 ± 0.07	94.78 ± 0.07	6.47 ± 0.01 f	9.31 ± 0.02 e
Ss.	*	ns	ns	*	*

(V - variant; G1 - genotype 1, …., G10 - genotype 10; Ss. - statistical significance: * significative, ns – insignificant; Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$).

A mature tomato fruit consists of approximately 90-95% water, with the remaining 5-10% being dry matter, predominantly carbohydrates (Wang et al., 2011). Rusu et al. (2023) observed that the water content of tomatoes varies depending on the cultivar, ranging from 93.59% to 94.03%. In our study, water content varied from 64.61% in cultivar G4 to 95.76% in cultivar G1. In the conventional cultivation system, water content ranged from 94.18% (G4) to 96.20% (G6), while in the organic system, it ranged from 93.83% (G2) to 95.81% (G1). Additionally, Kondratyeva and Molchanova (2022) reported that the water content of four tomato genotypes ranged between 92.5% and 95.1%.

Tomatoes and their derivatives are abundant in carotenoids, including lycopene, ascorbic acid, and phenolic compounds, all of which contribute to their nutritional value, colour, and flavour. Genetics, ripeness, and environmental factors all affect tomato composition (Shah et al., 2015). The carotenoid content, particularly lycopene, is a critical determinant of tomato quality. Studies have shown that the concentration of lycopene varies significantly with the maturity of the fruit, with mature tomatoes exhibiting higher levels compared to immature ones (Park et al., 2018). Additionally, the cultivar plays a crucial role in determining carotenoid profiles; for instance, certain cultivars like cherry tomatoes have been reported to possess higher levels of β-carotene and total carotenoids compared to standard varieties (Figàs et al., 2015).

Environmental factors, such as salinity and fertilization, also influence carotenoid accumulation, demonstrating the complex interplay between genetics and growing conditions (Paolo et al., 2018). Carotenoid concentrations have been shown to vary significantly among different tomato cultivars, with traditional varieties in particular offering a broad source of genetic diversity (Cortés-Olmos et al., 2014; Kavitha et al., 2014). Pandurangaiah et al. (2020) demonstrate that among the tomato lines was a significant difference in the total carotenoid content. The dark red fruit contained the highest carotenoid content with 23.80 mg/ g^{-1} F.W. The lycopene content ranged between 0.85 and 15.10 mg/ g^{-1} F.W. The least amount of carotene content was 0.80, and the highest content was represented by 8.56 mg/ g^{-1} F.W. In our study, depending on the cultivar, the lycopene content values ranged between 7.60 (G9) and 9.09 mg/g^{-1} F.W. (G2), and those in beta carotene ranged between 11.35 (G9) and 13.98 mg/g^{-1} F.W. (G2).

From a statistical point of view, no significant differences were observed between the results. The conventional system showed statistically significant differences in both lycopene and beta carotene content.

The highest levels for both compounds were found in the G2 genotype, with a maximum of 8.32 for lycopene and 12.49 mg/ g^{-1} F.W for beta carotene. On the other hand, the G9 genotype exhibited the lowest lycopene content at 5.95 mg/ g^{-1} F.W, while the G7 genotype had a beta carotene content of 8.39, which was statistically similar to G9's value of 8.43 mg/ g^{-1} F.W. In the organic system, the obtained values were noticeably higher. The highest values were achieved by G2 (9.85) and G8 (9.76), which were statistically similar, while the lowest values were 15.46 and 15.35, respectively.

CONCLUSIONS

The selection of cultivar is a critical factor in shaping the quality characteristics of tomatoes. Furthermore, the genetic composition of tomato cultivars plays a significant role in the accumulation of beneficial compounds, such as carotenoids and phenolic compounds, which are vital for both nutritional value and sensory appeal. Regardless of the crop system or

genotype studied, the highest values in terms of plant height and diameter at the base were obtained by the G5 genotype, an indeterminate cultivar, and the lowest values were obtained by the G10 genotype.

When focusing solely on the cultivar, the genotype with the longest time to fruit ripening is G4, taking approximately 117 days, while the earliest is the determinate growth genotype, G9, at 109.5 days. In the conventional system, the genotype with the shortest ripening period is G9, at 104.67 days, while the longest period is observed in G2, which takes 118.67 days. In the organic system, G1 is the most suitable genotype in terms of the earliest appearance of ripe fruits, taking 112.33 days, while G2 again has the longest period at 119 days.

Variation in fruit morphology is a prevalent characteristic among cultivated tomato. Regarding the organic system and the cultivars studied, the genotypes with the highest values obtained regarding the biometric measurements of the fruits were G5 for fruit diameter, G4 for fruit length and G5 for fruit weight. For the conventional system the maximum values were reached by G5 for diameter, and G4 for fruit length and fruit weight.

When examining the physiological characteristics according to cultivar, significant differences were observed in total soluble solids (TSS). The G2 cultivar achieved the highest value, indicating firmer fruits with a longer storage life, while G9 exhibited the lowest TSS value. No significant differences were found in dry matter, water percentage, beta carotene, or lycopene content across the cultivars.

Regarding the growing system, in organic cultivation, the G2 cultivar performed best in terms of the qualitative traits studied, while G9 and G10 had the lowest values for TSS, and G6 had the lowest for both lycopene and beta carotene. In conventional cultivation, no significant differences were found for dry matter and water percentage. However, the highest TSS and lycopene values were seen in G2, while G10 had the lowest for TSS and lycopene, and G9 had the lowest for beta carotene.

Preliminary analyses revealed the variability among accessions for all the evaluated traits, providing criteria to identify landraces that were less adapted to greenhouse conditions or that responded poorly to the management practices. This information will help in selecting the varieties to be included in the next experimental trial.

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THE INFLUENCE OF FERTILIZERS ON THE DEVELOPMENT OF THE ROOT SYSTEM AND PRODUCTION CAPACITY OF SWEET PEPPER (*CAPSICUM ANNUUM* L.)

Claudia BÎLĂIȚĂ[1,2], Dan Ioan AVASILOAIEI[2], Andreea ANTAL TREMURICI[2], MarianaCALARA[2], Gabriel-Alin IOSOB[2], Elena Maria DRĂGHICI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Vegetable Research and Development Station Bacău, 220 Calea Bârladului Street, Bacău, Romania

Corresponding author emails: avasiloaiei_dan_ioan@yahoo.com, draghiciem@yahoo.com

Abstract

The study investigates the impact of different fertilization strategies on the root development and yield capacity of Capsicum annuum *L. Four fertilization variants were tested: V1 - Graundfix®, V2 - Albit®, V3 - Rerum and V4 - Unfertilized (control) on three cultivars in polyethylene greenhouse soil. Parameters assessed included plant height, stem collar diameter, root length, root width and weight, and yield per plant. The application of biofertilizers (V1 and V2) demonstrated a positive impact on the root system activity of the greenhouse culture, when compared to the control and chemical fertilizer treatments. The productivity of the plants was found to be directly proportional to the root parameters.*

Key words: *root system, fertilizer, productivity, sweet pepper.*

INTRODUCTION

Sweet peppers are a popular vegetable consumed worldwide, with a wide range of cultivation regions contributing significantly to the global supply. Both for domestic consumption and for export, Romania produces a significant quantity of sweet peppers.

Crops are predominantly cultivated in open fields; however, there is an emerging trend towards protected crops, such as greenhouse crops, with the objective of extending the growing season and enhancing crop yield and quality.

Research endeavours are currently underway to enhance crop yields, despite the presence of various adverse environmental factors. A multifaceted approach, encompassing physical, chemical, and biological methods, is being employed to address the damage caused by biotic and abiotic stresses on plant life (Radhakrishnan et al., 2017).

The root system of sweet pepper plants is essential for their growth, health and productivity. Understanding the characteristics and functions of the root system can help improve growing practices. Pepper plants, including sweet peppers, have a relatively fibrous root system in the topsoil (0-30 cm), even if the root is initially tap-rooted (Figure 1a, b) (Teliban et al., 2020). The primary root develops from the seed and grows downwards, while secondary and tertiary roots branch out laterally and spread horizontally. (Figure 1)


a b

Figure 1. The root of *Capsicum*
(a - tap root; b - fibrous root)

The root system of pepper plants is usually not very deep compared to other plant species, and adventitious roots develop from the hypocotyl zone. These roots are designed to facilitate efficient uptake of nutrients and water from the soil to support plant growth and fruit

production. Some roots can store carbohydrates and other nutrients for use in times of stress or when above-ground growth is limited. Roots interact with soil micro-organisms, including beneficial bacteria and fungi, which can increase nutrient availability and protect against pathogens.

In the contemporary era, agricultural practices are subject to various factors that can impede their efficacy, including but not limited to declining soil fertility, a deficiency of nutrients within the soil, or the presence of pests. In order to address these challenges, modern agriculture relies on the utilization of fertilizers, with the objective of enhancing yield production to meet the escalating demands of a growing population. This approach encompasses the application of both chemical and biological fertilizers, which play a pivotal role in augmenting agricultural productivity and enhancing plant tolerance to diseases and pests. While chemical fertilizers contribute to increased productivity, their prolonged use can result in the alteration of the physico-chemical and biological characteristics of the soil (Pahalvi et al., 2021).

Fertilizers have been shown to enhance plants' capacity to retain moisture and increase root depth. The phosphorus present in fertilizers has been demonstrated to promote accelerated root growth (ALnaass et al., 2021).

Chemical fertilizers have been demonstrated to augment crop yield by supplying essential nutrients; however, they can also lead to significant environmental concerns. Consequently, there has been a growing interest in biofertilizers due to their environmentally friendly and pollution-free characteristics. (Gou et al., 2020).

Biofertilizers are defined as materials containing natural biocompounds, which are constituted of microorganisms that, when applied to seeds, plant surfaces, or soil, promote plant growth and development by increasing the supply or availability of primary nutrients to the host plant (Calara et al., 2020). The utilization of these products has a positive impact on both the environment and the crop by increasing plant productivity (Rati et al., 2015; Raducanu et al., 2016). These biofertilizers may contain micro- or macro-organisms, plant residues, enzymes, or substances that enhance plant growth by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances. These products are an environmentally friendly alternative to chemical fertilizers and play a key role in sustainable agriculture (Nosheen et al., 2021).

The primary objective of the research endeavour is to observe and document the behaviour of pepper roots under conditions of protected spaces, and subject to the influence of both chemical and biological fertilisers.

MATERIALS AND METHODS

The experiment was conducted at Vegetable Research and Development Station Bacau during the 2024 season. Three cultivars of sweet peppers were utilized: Dariana Bac (C1), Dariochea (C2), and Matiusca (C3). These peppers were obtained from the research station.

In this study, the three sweet pepper cultivars C1, C2 and C3 were cultivated in polyethylene greenhouse soil under three different fertilizations: V1 - Groundfix, V2 - Albit®, V3 - Rerum, and V4 - Unfertilized (control). The objectives of this study were twofold: firstly, to examine the effects of fertilization on root morphological parameters, and secondly, to detect the relationships between root morphological parameters and the three cultivars, in agreement with pepper yield.

Seedlings of sweet pepper were transplanted in the field at age of 55 days in the second week of April in 2024. Sweet pepper seedlings were planted in furrows measuring 70 centimeters in width, situated between rows, with a separation of 25 centimeters on soil that had been mulched.

The fertilization process was executed in two stages, with the initial stage occurring one week after planting and the subsequent stage occurring one month after the first. The experimental design incorporated two organic root fertilizers, Graundfix® and Albit® (V1 and V2 variants, respectively), along with a chemical fertilizer, Rerum, administered through foliar application.

The Graundfix® product has been demonstrated to optimize the availability and mobility of phosphorus and potassium in the

soil, thereby enhancing the efficiency of nitrogen fixation. It has been shown to have the capacity to substantially improve crop yield and reduce environmental impact by increasing the nutrient utilization rate of natural fertilizers by a factor of 1.2 to 1.5. Additionally, it facilitates the free flow of silica in plants, stimulates biological processes in the soil, and contributes to soil health, thus preventing soil degradation. The product contains cells of the bacteria *Bacillus subtilis*, *Bacillus megaterium* var. *phosphaticum*, *Enterobacter spp*., *Azotobacter chroococcum*, *Paenibacillus polymyxa*. These bacteria are involved in a number of physiological and molecular mechanisms that enable plants to cope with environmental stressors such as extreme temperatures, water shortage, or exposure to toxic substances.

Albit® is a biostimulator that contains poly-beta-hydroxybutyric acid (a natural biopolymer synthesized from beneficial bacteria such as *Bacillus megaterium* and *Pseudomonas aerofaciens*) and a set of substances including urea, potassium nitrate, magnesium sulfate, potassium phosphate, and conifer extract. The functionality of Albit® is multifaceted, encompassing the stimulation of seed germination, the promotion of robust root development, and the enhancement of nutrient uptake from the soil. It has been observed to stimulate the growth and development of plants, thereby increasing their resilience to extreme temperatures, diseases, and pests. Additionally, Albit® has been demonstrated to improve crop yields, and it is non-toxic and harmless to bees.

Rerum has been demonstrated to exhibit auxin-like characteristics in the context of physiological processes in plants. The product is systemic and is known to enter the absorptive complex of the plant in close temporal proximity to application. It contains the complete range of amino acids that are essential for plant nutrition and is particularly noteworthy in its capacity to enhance the quantity and quality of agricultural production. It has been observed to accelerate the assimilation of inorganic nitrogen, while concurrently enhancing plant resistance mechanisms to diseases and pests. It is an indispensable precursor in auxin synthesis.



Figure 2. Foliar fertilizer application

The plants were individually measured to determine plant root and stem length. The analysis of pepper root morphology was conducted by first severing the roots from the base of the stem, followed by meticulous measurement of their width and length (Figure 3).



Figure 3. Root width measurement

The root morphology parameters, including total plant height (TPH), stem collar diameter (SCD), root length (RL), root width (RW) and root weight (RWE). The mean productivity (PR) was computed for each of the three fertilizers, as well as for the control sample. The results are expressed in tons per hectare (t/ha).

The statistical interpretation was executed with the utilization of SPSS software, version 21. This software facilitated the implementation of analysis of variance (ANOVA) for 95% confidence and the Tukey test to ascertain the significance of observed differences.

RESULTS AND DISCUSSIONS

It has been demonstrated that plants possess the capacity to detect resources present within the soil, in addition to the presence of foreign roots. In response to these stimuli, plants adapt their root development. A study conducted by researchers at Princeton University observed the behaviour of the roots of pepper plants grown singly or in pairs. It was observed that the farther consumes to transport organic molecules. Furthermore, the plant develops a series of vessels capable of transporting water and nutrients to the stem. Consequently, in the presence of competitors, the plant reduces the extent of its root system, but develops an over density of roots close to its stem (Butor, 2021).

A well-developed root system can have the benefit of supporting accelerated plant growth in the early stages of crop growth. It can also facilitate the extraction of water from shallow soil layers, which is otherwise susceptible to loss through evaporation (Kulkarni & Phalke, 2009).

The importance of root system size has long been recognised as being critical in the management of drought conditions (You et al., 2025).

Results of morphological parameters in pepper root according to the fertilizer type used and the yield are shown in figure 4-8.

When monitoring the total plant height (Figure 4), no significant differences are observed between the variants, but a slight increase is observed in the case of Biofertilizer V2 (115.63 cm)

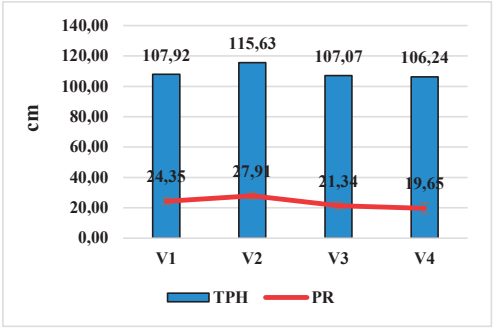


Figure 4. Comparison of Total Plant Height (TPH) and productivity (PR)

Subsequent to the implementation of the fertilizers, a balance was achieved in the diameter of the collar stem (Figure 5). It was observed that all three fertilization variants, in conjunction with the control, exhibited equivalent values.

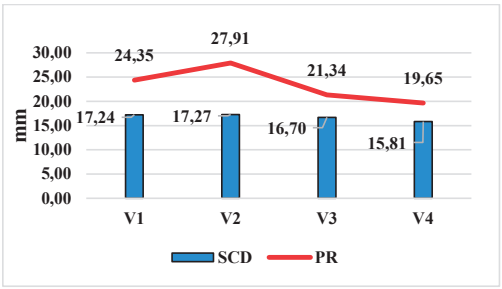


Figure 5. Comparison of Stem Collar Diameter (SCD) and productivity (PR)

Root length is a key indicator of the ability of a plant to take up water from deeper layers of the soil, and it is influenced by an increase in root penetration (Kulkarni & Phalke, 2009).

The fertilization variants V2 (21.27 cm) and V3 (21.17 cm) resulted in an increase in the measured root length, while V1 demonstrated a positive correlation with V4 (Figure 6).

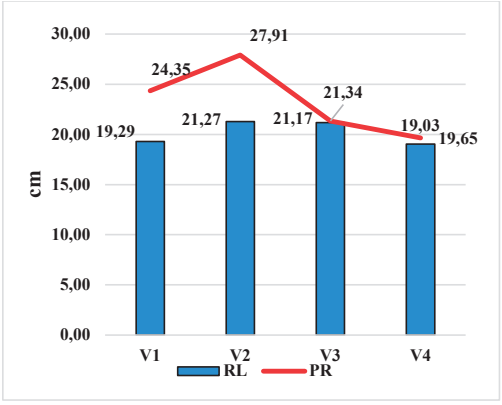


Figure 6. Comparison of Root Length (RL) and productivity (PR)

The impact of the fertilizer was most pronounced in terms of root width in the V2 sample (36.86 cm), followed by the V1 sample, the V3 sample, and finally the V4 sample (Figure 7).

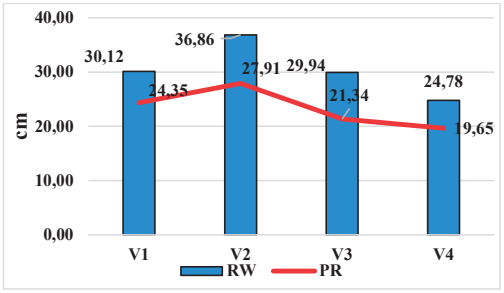


Figure 7. Comparison of Root Width (RW) and productivity (PR)

In the case of root weights, a slight increase was observed for V1 fertilizer, but not an increase in productivity for the same variant (Figure 8).

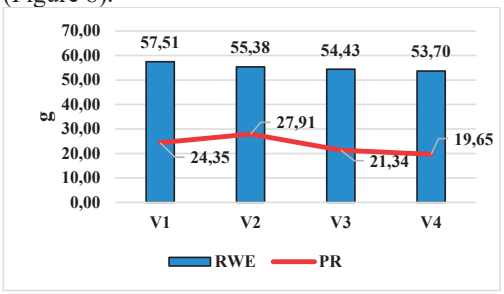


Figure 8. Comparison of Root Weight (RWE) and productivity (PR)

Thus, it is observed that all the three fertilizers used caused an increase in the analysed parameters compared to the control sample.

The observed productivity trajectory was consistent across all five parameters under analysis. The highest values were obtained for V2 fertilizer (27.91 t/ha), followed by V1, and then by V3 and V4.

As illustrated in Figure 9, the analysis encompasses all parameters evaluated for each cultivar. The graph reveals that each cultivar exhibits distinct patterns of root development. Cultivar C1 demonstrates higher values for the parameters of root length (22.04 cm) and root width (31.68 cm). Cultivar C2 exhibits average values for all five parameters, while cultivar C3 shows elevated values for total plant height (146.15 cm), stem collar diameter (18.53 mm) and root weight (66.36 g).

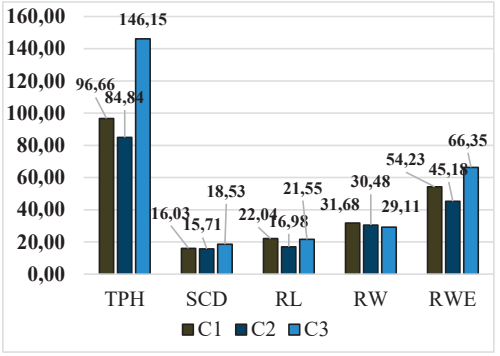


Figure 9. Correlations between cultivar types and analyzed parameters (TPH - total plant height; SCD - steam collar diameter; RL - root length; RW - root width; RWE - root weight)

Table 1. Results of root morphological characteristics and productivity according to cultivar and fertilizer type (2024)

	TPH (cm)	SCD (mm)	RL (cm)	RW (cm)	RWE (g)	PR (T/ha)
C1xV1	85.02±4.68 ab	25.59±3.25 ns	23.54±1.51 a	19.85±2.84 ns	63.05±1.05 ab	28.23±3.46 ns
C1xV2	94.66±0.55 ab	20.92±2.72 ns	25.13±0.36 a	29.46±6.82 ns	46.27±1.02 b	23.41±3.47 ns
C1xV3	82.17±4.62 ab	25.00±5.68 ns	21.40±0.89 ab	19.37±2.33 ns	43.87±2.70 b	24.82±1.77 ns
C1xV4	82.55±9.88 ab	35.27±7.46 ns	22.63±0.67 a	23.39±3.66 ns	84.28±8.50 a	29.03±3.31 ns
C2xV1	77.27±1.28 ab	21.44±3.06 ns	22.61±1.30 a	23.33±5.16 ns	60.24±8.32 ab	24.32±2.10 ns
C2xV2	87.97±7.87 ab	23.84±2.59 ns	21.91±1.91 ab	26.12±5.38 ns	53.53±6.04 ab	29.82±1.85 ns
C2xV3	82.35±0.94 ab	25.61±3.27 ns	24.82±1.87 a	25.87±5.08 ns	66.61±2.87 ab	19.83±2.91 ns
C2xV4	65.00±4.52 b	23.25±5.11 ns	13.45±1.65 b	13.31±0.70 ns	42.67±13.82 b	25.53±5.25 ns
C3xV1	119.56±15.32 a	27.05±3.72 ns	28.13±4.56 a	21.63±3.49 ns	67.92±1.10 ab	29.70±5.14 ns
C3xV2	107.98±17.62ab	24.87±2.72 ns	23.99±1.17 a	20.25±3.35 ns	68.47±7.99 ab	33.38±4.51 ns
C3xV3	104.54±17 ab	25.22±2.56 ns	22.08±0.78 a	19.92±3.68 ns	82.92±7.97 a	30.07±6.20 ns
C3xV4	104.86±15.28ab	30.74±6.14 ns	23.59±0.20 a	22.91±3.71 ns	70.72±5.97 ab	36.73±8.79 ns

*The values represent the mean parameters ± standard error; the lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a, b - represents the highest value; ns - not significant).
C1 - Dariana Bac cultivar; C2 - Dariochea cultivar; C3 - Matiusca cultivar;
V1 - Groundfix fertilizer; V2 - Albit fertilizer; V3 - Rerum fertilizer; V4 - Unfertilizer (control)

As illustrated in Table 1, the impact of cultivar and fertilizer interaction on the parameters under observation is evident. The range of total plant height varies from 119.56 cm for C3 x V1 to 65.00 cm for C2 x V4, while stem collar diameter demonstrates a maximum value of 35.27 mm in C2 x V4 and a minimum value of 20.92 mm in C1 x V2. The average value of root length ranges from 13.45 cm in C2 x V4 to 28.13 cm in C3 x V1. The diameter of the roots ranges from 13.31 cm in C2 x V4 to 29.46 cm in C1 x V2, and the weight of the roots ranges from 42.67 g in C2 x V4 to 84.28 g in C1 x V4. Yield ranges from 19.83 tons per hectare in C2 x V3 to 36.73 tons per hectare in C3 x V4.

CONCLUSIONS

The present study demonstrated that the relationship between pepper crop productivity and root morphology was predominantly influenced by the type of fertilizer utilized, particularly its composition. Higher productivity values were obtained with biofertilizers due to the presence of microorganisms.

Plants have developed adaptive strategies that enable them to activate different signalling and response pathways, thereby facilitating survival and thriving in adverse conditions. A comprehensive understanding of these mechanisms is imperative for the development of innovative technologies that can support sustainable agriculture and ensure global food security.

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GROWING ONION IN ROMANIA: CHALLENGES AND OPPORTUNITIES

Elena BARCANU TUDOR, Ovidia Loredana AGAPIE, Ion GHERASE, Eliza TEODORESCU

Vegetable Research and Development Station Buzău, 23 Mesteacănului Street, 120024, Buzău, Romania

Corresponding author email: teodorescu.eliza@gmail.com

Abstract

This research examines the current status of Allium cepa (onion) cultivation in Romania, highlighting its significance for food security and sustainable agriculture. Despite Romania's rich agricultural heritage and favourable conditions, onion production faces challenges like outdated practices, reliance on imported seeds, and inefficient supply chains. Although Romania ranks sixth in the EU for onion cultivation area, domestic consumption often exceeds production, leading to increased imports. The study explores opportunities to enhance yield and quality through modern breeding techniques, technological advancements, and international collaborations. It also underscores the need for preserving native onion landraces to maintain genetic diversity and promote resilience against climate change. Strengthening agricultural infrastructure, improving organization among farmers, and optimizing supply chains are critical for reducing market imbalances and waste. An integrated approach combining scientific research, sustainability principles, and market knowledge is essential to unlock Romania's onion industry potential, support rural livelihoods, and contribute to national food security and economic growth.

Key words*: Allium cepa, landrace, sustainable cultivation.*

INTRODUCTION

Onions (*Allium cepa* L.) hold a pivotal role in global agriculture as one of the oldest and most versatile cultivated crops. Native to southwestern Asia, onions have spread worldwide, becoming integral to culinary traditions and food systems across cultures (Devi et al., 2015; Rabinowitch and Currah, 2002). Their significance lies not only in their culinary uses but also in their resilience as a crop suitable for storage, transport, and processing into value-added products like powders and flakes. Furthermore, onions are a crucial source of bioactive compounds, such as flavonoids and sulfur-containing compounds, which contribute to human health (Griffiths et al., 2002).

Globally, onion production exceeded 110 million tons in 2022, with India and China as the largest producers, contributing nearly half of the total output (FAOSTAT, 2022). Within the European Union, Romania ranks sixth in onion cultivation area, with 16,300 hectares dedicated to the crop in 2021 (Eurostat, 2021). Despite this, the country struggles to meet domestic demand, importing significant quantities to fill the gap (KeysFin, 2022). This paradox highlights inefficiencies in the supply chain, limited adoption of modern agricultural practices, and a heavy reliance on imported hybrid seeds.

Romania’s rich agricultural heritage includes the cultivation of traditional onion varieties, locally known as landraces, which are valued for their adaptability and contribution to biodiversity (Străjeru et al., 2009). However, the rise of imported hybrid seeds and the decline of local seed production pose a threat to the preservation of this genetic diversity. Climate change, fluctuating market demands, and insufficient infrastructure further exacerbate these challenges, making it difficult for farmers to achieve consistent yields and quality (Hammer & Laghetti, 2005; Sarli et al., 2016).

This study investigates the current status of onion cultivation in Romania, examining its economic, cultural, and environmental significance. The research provides actionable insights to strengthen Romania's onion industry and enhance its contribution to food security and economic growth.

MATERIALS AND METHODS

This study employed a multidisciplinary approach to analyse the status of onion cultivation in Romania, focusing on production practices, economic impact, and sustainability. The methodology combined an in-depth review of literature, statistical analysis, and an assessment of ongoing research efforts.

Statistical data were collected from reputable sources, including Eurostat, FAOSTAT, and the Romanian National Institute of Statistics (INS, 2021, Romanian Statistical Yearbook, 2020), to evaluate trends in onion production, cultivated areas, and consumption patterns. These datasets provided valuable insights into the agricultural sector's structure and performance at the national and European levels. Additional economic insights were gathered from reports by the Ministry of Agriculture and Rural Development and independent market analysis firms, such as KeysFin.

A thorough review of peer-reviewed articles, research reports, and contemporary data sources was conducted to understand the broader context of onion cultivation, focusing on breeding programs, landrace conservation, and climate resilience.

The Vegetable Research Development Stations (VRDS) in Bacău, Buzău, and Iernut are currently involved in a collaborative project aimed at revitalizing and improving onion seed varieties in Romania. While results from these studies are not yet published, these initiatives are crucial for addressing key challenges, such as genetic erosion and the development of climate-resilient onion varieties. This research acknowledges the ongoing efforts of these institutions and their importance in shaping the future of Romanian vegetable sector.

RESULTS AND DISCUSSIONS

Romania's onion cultivation is a testament to its agricultural heritage and ongoing research efforts to modernize the sector. The State Institute for Testing and Registration of Varieties (ITRV) lists 15 onion genotypes in its official 2024 catalogue, comprising 12 seed-propagated varieties (Figure 1) and 3 bulb-propagated types (Official Catalogue of Crop Plants in Romania, 2024).

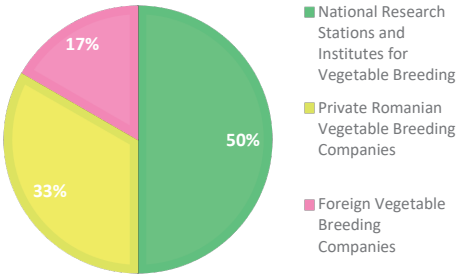


Figure 1. Origin of onion seed cultivars and hybrids listed in the ITRV Catalogue

Among the seed-propagated varieties, 6 were developed by national research institution and stations, including the Vegetable Research and Development Stations (VRDS) in Bacău (Orizont), Buzău (De Buzău and Rubiniu), and Iernut (Arieșana and Chibed), as well as the Institute of Vegetable Breeding Vidra (Universal de Vidra). These varieties have been meticulously bred over decades to improve yield, disease resistance, and adaptability to diverse environmental conditions in Romania.

The remaining six seed-propagated varieties include two hybrids developed by an Italian seed company and three varieties (one hybrid and two open-pollinated types) created by a private Romanian seed company. While these hybrids are valued for their high yield potential and disease resistance, they require higher levels of inputs, including fertilizers and irrigation, and are often less adaptable to local conditions than traditional varieties.

Romania's research stations have historically been instrumental in the development and testing of certified seeds, particularly onion varieties tailored to local conditions. However, in recent decades, these institutions have faced significant challenges due to the loss of much of their agricultural land. Once managing extensive acreage dedicated to seed production and field trials, these stations now operate with limited areas for cultivating seed material. Consequently, the production of certified onion seeds has declined, and supply often fails to meet market demand. This gap has contributed to an increased reliance on imported hybrid seeds, limiting the availability of locally bred varieties and raising concerns about genetic erosion (Hammer & Laghetti, 2005; Străjeru et al., 2009).

The conservation of onion (*Allium cepa* L.) landraces remains a cornerstone of sustainable agriculture in Romania, where traditional agro-ecosystems have historically served as vital repositories of genetic diversity. Landraces, which are locally adapted varieties developed over centuries, are invaluable for their resilience to pests, diseases, and environmental stressors. Romania's onion landraces, such as De Buzău, exemplify this genetic richness, offering traits that are increasingly critical in the face of climate variability (Străjeru et al., 2009; Brush & Stabinsky, 1996).

However, the rise of hybrid seeds and the decline of traditional farming practices have accelerated biodiversity loss globally. Esteemed scientists, including Harlan (1972), Frankel et al. (1970), and Hammer and Laghetti (2005), have warned of the erosion of genetic diversity and the displacement of landraces by genetically uniform, high-yielding cultivars. These trends are not limited to onions but extend to many crops essential to global food security.

In Romania, landrace conservation efforts have been hindered by declining vegetable-cultivated areas (Ibănescu et al., 2002), the advanced age of seed producers (71% of whom are over 60 years old), and underfunding of research centers. Additionally, traditional knowledge associated with landrace cultivation is at risk of disappearing, further threatening these genetic resources. Programs like ADER 6.3.6 aim to address these challenges by emphasizing the preservation and use of landrace germplasm in breeding programs. By integrating traditional genetic resources into modern agricultural systems, these initiatives can help safeguard Romania's agricultural biodiversity while supporting sustainable agricultural development in Eastern Europe.

Onion production in Romania faces a myriad of challenges, many of which stem from the country's fragmented agricultural landscape. According to Eurostat (2015), 97% of Romania's agricultural holdings are small-scale farms of less than 10 hectares, with an average size of 3.3 hectares per farm. This fragmentation limits economies of scale, making it difficult for farmers to invest in modern technologies, irrigation systems, and storage infrastructure (Rusu et al., 2011). The prevalence of subsistence and semi-subsistence farming further exacerbates inefficiencies, as many smallholders prioritize production for personal consumption rather than for commercial markets.

While traditional breeding and hybrid development have been the primary methods for onion improvement in Romania, modern biotechnological tools offer promising avenues for increasing productivity and sustainability. Advancements in onion (*Allium cepa* L.) breeding have significantly improved yield potential, disease resistance, and adaptability to climate change. Traditional breeding methods, such as backcross breeding and hybridization, have been widely used to enhance traits like bulb size, storage life, and pest resistance (Singh et al., 2018). However, modern biotechnological approaches are now revolutionizing onion breeding.

Recent research has focused on marker-assisted selection (MAS) and genome-editing techniques like CRISPR-Cas9 to accelerate breeding cycles and introduce targeted improvements (Khosa et al., 2016). These techniques allow for precise modifications in genes responsible for disease resistance and stress tolerance, reducing dependency on chemical inputs.

The development of male-sterile lines has facilitated hybrid onion varieties with higher yield potential and uniformity (Manjunathagowda et al., 2021). Hybrid onions, while requiring more inputs, have shown greater resistance to pests and diseases, making them more suitable for large-scale commercial production.

Tissue culture methods, such as somatic embryogenesis and meristem culture, are being utilized to produce disease-free planting material (Singh & Khar, 2021). This technique ensures uniform plant growth and speeds up the multiplication of elite onion varieties, particularly those with desirable agronomic traits.

Technological advancements, including remote sensing, automated irrigation, and artificial intelligence (AI)-driven crop monitoring, are helping optimize resource use in onion cultivation (Ochar and Kim, 2023). GPS-guided planting and drone-assisted pest control are being increasingly adopted to improve efficiency and reduce environmental impact.

While these technologies are widely used in developed agricultural markets, their adoption in Romania remains limited due to financial constraints and the predominance of small-scale farming.

Romania's continental climate, characterized by seasonal extremes and irregular precipitation, poses additional risks to onion cultivation. High summer temperatures often lead to water stress, resulting in smaller bulb sizes and reduced yields, while late spring frosts can damage seedlings and delay planting schedules (Rabinowitch & Currah, 2002; Devi et al., 2015). Despite the fertility of Romania's Chernozem soils, which are well-suited for onion cultivation (Stănilă et al., 2011), the lack of irrigation infrastructure leaves farmers vulnerable to prolonged droughts and heavy rains, both of which have become more frequent due to climate change (Sarli et al., 2016).

Post-harvest losses present another significant challenge. According to KeysFin (2022), a substantial proportion of domestically produced onions is lost due to inadequate storage facilities, poor transportation networks, and inefficient supply chains. Modern cold storage systems, sorting equipment, and transport logistics are essential for preserving bulb quality and extending shelf life, yet such infrastructure remains underdeveloped across much of the country.

Unlike Romania, where cold storage facilities remain underdeveloped, the Netherlands has invested in precision-controlled storage technologies, significantly reducing onion losses - by up to 30% in some cases (Currah & Cools, 2012). Adopting similar measures could improve Romania's onion market stability and reduce post-harvest losses. Smart cold storage solutions, controlled atmosphere storage, and blockchain-based supply chain management are improving onion preservation and reducing market fluctuations (Sidhu et al., 2004).

Despite these challenges, Romania's onion sector has immense potential for growth and revitalization. With 16,300 hectares dedicated to onion cultivation in 2021, Romania produced approximately 357,000 tons of onions, ranking as the sixth-largest producer (5.7%, Figure 2) in the European Union (Eurostat, 2021).

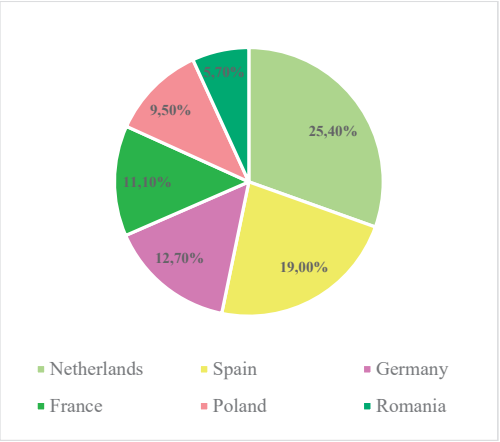


Figure 2. Percentage of EU onion production

However, domestic demand exceeds production, with consumption reaching 400,000 tons annually, resulting in imports worth 40 million euros in 2021 (KeysFin, 2022). Addressing this supply-demand gap requires a multifaceted approach, including investments in research, infrastructure, and sustainable farming practices.

Breeding programs led by VRDS Buzău, Bacău, and Iernut, as well as Institute of Vegetable and Floriculture Breeding Vidra, have developed cultivars such as De Buzău, Rubiniu, Orizont, Arieșana and Chibed and Universal de Vidra, which are tailored to Romania's agro-climatic conditions. Expanding the capacity of these research centers to produce certified seeds could help reduce reliance on imported hybrids, which often require high inputs and are less adapted to local conditions (Străjeru et al., 2009). Additionally, the ADER 6.3.6 program offers a promising framework for developing climate-resilient onion varieties by leveraging Romania's genetic diversity.

Modernization of agricultural practices is also critical. Precision agriculture technologies, such as automated irrigation systems and GPS-guided machinery, can optimize resource use and reduce production costs. Integrated pest management (IPM) strategies can further enhance sustainability by reducing the reliance on chemical inputs (Rabinowitch & Currah, 2002). Improving post-harvest infrastructure is another priority.

Investments in cold storage facilities and mechanized sorting systems can reduce losses, improve product quality, and enable better access to both domestic and export markets. Furthermore, the formation of farmer cooperatives could help smallholders pool resources, access financing, and strengthen their bargaining power with retailers, ultimately improving market organization and reducing inefficiencies (Agarwal et al., 2021).

CONCLUSIONS

Romania's onion cultivation sector exemplifies the intricate balance between tradition and modernization in agriculture. Despite its historical significance and favourable agro-climatic conditions, the country faces systemic challenges that limit the full potential of onion production. Key issues include outdated farming practices, insufficient post-harvest infrastructure, and heavy reliance on imported hybrid seeds, all of which threaten the sustainability and competitiveness of the sector. Efforts to address these challenges must prioritize the modernization of agricultural practices, including the adoption of precision technologies, the establishment of farmer cooperatives, and investments in cold storage and processing facilities. Additionally, policy frameworks should incentivize local seed production to preserve native onion landraces, which are vital for maintaining genetic diversity and fostering resilience against climate change.

The ADER 6.3.6 program and similar initiatives offer promising pathways for sustainable development by integrating advanced breeding techniques and biodiversity conservation. Collaborative research at national and international levels is essential to develop climate-resilient onion varieties that can withstand the environmental variability characterizing Romania's agricultural landscape.

Market organization remains a critical area for improvement. Strengthening supply chains, reducing waste, and ensuring consistent pricing mechanisms will bolster both farmer incomes and consumer satisfaction. By addressing these systemic inefficiencies, Romania can reduce its dependency on imports, improve its self-sufficiency, and position itself as a competitive player in the European onion market.

Sustainability must remain at the core of future strategies, encompassing environmentally friendly practices, resource-efficient technologies, and the preservation of Romania's rich agricultural heritage. To secure Romania's onion industry for the future, integrating cutting-edge breeding techniques, improving post-harvest infrastructure, and expanding farmer support programs will be essential. Strengthening policies that encourage local seed production and investing in sustainable storage solutions can help reduce reliance on imports, improve competitiveness, and enhance food security.

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IDENTIFYING CULTIVARS FOR ORGANIC FARMING: A CASE STUDY ON SOLANACEOUS VEGETABLES IN ROMANIA

Elena BARCANU TUDOR , Ion GHERASE, Ovidia Loredana AGAPIE, Eliza TEODORESCU

Vegetable Research and Development Station Buzău, 23 Mesteacănului Street, 120024, Buzău, Romania

Corresponding author email: ion2196@yahoo.com

Abstract

Organic farming is a key priority for the European Union, aiming for 25% of agricultural land under organic cultivation by 2030. In Romania, organic farming expanded by 67% between 2012 and 2022, increasing the need for cultivars suited to non-chemical systems. This study evaluates eleven tomatoes (Solanum lycopersicum), five pepper (Capsicum annuum), and five eggplant (Solanum melongena) cultivars, traditionally bred for conventional farming, assessing their yield, fruit quality, and adaptability under organic conditions in southern Romania. Results indicate that Darsirius tomato maintained stable yields (2.85 kg/plant organic vs. 3.05 kg/plant conventional), while Regal pepper exhibited stable productivity (140.16 g/fruit organic vs. 142.18 g/fruit conventional) and superior nutritional quality. Rebeca eggplant showed strong pest and disease resistance. Ema de Buzău recorded the highest dry matter content (10.82%), and Andrada had the highest lycopene concentration (12.7 mg/100g organic). These findings support selecting robust cultivars for organic farming, aiding sustainable agriculture and food security in Romania.

Key words*: Capsicum annuum, organic farming, sustainable agriculture, Solanum lycopersicum, Solanum melongena.*

INTRODUCTION

The overuse of chemical inputs in agriculture over the past century has led to severe environmental consequences, including soil degradation, climate change, and water contamination. In response, there is a growing shift toward ecological farming practices aimed at mitigating damage and promoting sustainability. Organic farming has become a key priority for the European Commission, with an ambitious target of converting at least 25% of agricultural land to organic cultivation by 2030 (European Commission, 2021).

Organic farming relies on natural fertilizers and pest control methods while restricting synthetic inputs, genetically modified organisms, and other non-natural practices (Willer et al., 2019). Across Europe, efforts to transition agriculture toward sustainability emphasize environmental responsibility, food quality, and social welfare (Fortea et al., 2022). In Romania, the organic sector is expanding rapidly due to supportive agricultural policies and increased consumer demand (Stoica et al., 2022). According to Eurostat, Romania ranked 9th in the EU for organic agricultural area in 2015, with organic farming covering 1.66% of total farmland - a figure that has since grown substantially (Eurostat, 2018).

With the expansion of organic agriculture, there is a rising need for crop cultivars optimized for nonchemical farming systems. Research indicates that plant phenotypes express differently under organic versus conventional conditions, necessitating dedicated breeding efforts (Lammerts van Bueren et al., 2011; Mason and Spaner, 2006; Reid et al., 2011; Wortman et al., 2013; Barcanu et al., 2020). The debate continues on whether organic-specific cultivars should be developed or whether conventional varieties can be adapted to organic conditions. A common approach to assessing adaptation is testing genotype-by-system interactions by cultivating conventional hybrids under organic management (Wortman et al., 2013).

At the Vegetable Research Development Station Buzău, we are evaluating selected tomato (*Solanum lycopersicum* L.), pepper (*Capsicum* spp.), and eggplant (*Solanum melongena* L.) cultivars bred for conventional farming to assess their performance under organic conditions. This research aims to

identify promising varieties that can support the transition to more sustainable agricultural practices.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at 45°08'60.00"N and 26°49'59.99"E, under a temperate climate with an average annual precipitation of 530 mm, approximately 60% occurring during the Solanaceae growing season. Weather conditions were monitored from May to October 2023 and compared to multiannual data. The crops were cultivated on sandy loam soil (pH 6.9), with 3.10% humus and 8.3% organic matter content.

Genotype Resources

Genotypes were sourced from the Vegetable Research and Development Station (VRDS) Buzău. The study included eleven tomato genotypes (*Solanum lycopersicum* L.) classified as indeterminate (Siriana, Ema de Buzău, Flaviola, Hera, Andrada), determinate (Măriuca, Darsirius, Chihlimbar, Kristinica, Florina), and semi-determinate (Ovidia). Additionally, five *Capsicum* spp. Cultivars - three sweet peppers (Regal, Cantemir, A50) and two hot peppers (Roial, Decebal) - along with five eggplant genotypes (*Solanum melongena* L.) (Rebeca, Iarina, Romanița, H13Bz, H2Bz) were included.

For differentiation, genotypes grown under organic conditions are denoted with 'O', while those cultivated in the conventional system are marked with 'C' throughout the study. Seedlings were started in alveolar trays with 50 cm^3 peat cubes and grown under controlled greenhouse conditions before transplanting in May. Plants were arranged in a randomized complete block design (70 cm inter-row, 30-35 cm intra-row spacing) with drip irrigation for efficient water and nutrient delivery. An innovative fence system using *Helianthus tuberosus* shoots supported pepper and indeterminate tomatoes, while windbreaks with corn, *Jerusalem artichoke*, and *Phaseolus* sp. were established.

Fertilization and Pest Management

The conventional system used chemical fertilizers (NPK 20-20-20, NP 15-50 + 2MgO, NPK 9-18-27+ 2MgO), while organic crops received four applications of NOV@ (21% organic carbon, 5% K_2O, 0.6% betaine) and foliar fertilization with Fylloton (*Ascophyllum nodosum*-derived). Pest management employed biocontrol agents (*Metarhizium anisopliae, Verticillium lecanii, Bacillus subtilis, Trichoderma* spp.) and companion planting with *Ocimum basilicum, Tagetes patula, Tropaeolum majus*, and others.

Fruit Quality Assessment

Fruit quality was evaluated for firmness (N), total soluble solids (TSS, °Brix), titratable acidity (TA, citric acid %), TSS/TA ratio, dry matter (%), lycopene, and β-carotene content. Peppers were analyzed at red maturity stages, while tomatoes and eggplants were assessed at harvest maturity. Five randomly selected fruits per genotype were analyzed in triplicate.

Analytical Methods

Dry Matter (%): Determined using AOAC 930.04 (2005).

Total Soluble Solids (TSS, °Brix): Measured via digital refractometer (KERN OPTICS ORF 1RS) following AOAC 932.12.

Lycopene & β-carotene (mg/100g): Extracted with hexane:acetone (4:6 v/v) and quantified spectrophotometrically (Nagata & Yamashita, 1992).

Firmness (N): Assessed using a TR Turoni 53205 electronic penetrometer.

Statistical Analysis

Statistical analysis was performed using ANOVA, with Duncan's test applied for mean comparisons.

RESULTS AND DISCUSSIONS

The 2023 growing season was characterized by higher mean temperatures and precipitation deficits, impacting crop performance. Climate variability highlights the importance of breeding resilient cultivars for organic farming (Brezeanu et al., 2020; 2022).

Solanaceous fruit yields in organic and conventional systems

Tomato yields varied significantly across genotypes and farming systems (Figure 1). Siriana had the highest yield in both conventional (4.64 kg/plant) and organic (4.16 kg/plant) systems, while Ovidia exhibited the lowest organic yield (2.25 kg/plant). No statistically significant differences were

observed between systems, though Darsirius maintained similar yields in both conditions (2.85 kg/plant organic vs. 3.05 kg/plant conventional).

These findings suggest that genotype selection plays a crucial role in ensuring stable yields across farming systems. Previous studies indicate that the yield gap between organic and conventional systems is largely genotype-dependent (Bàrberi, 2015; Murariu et al., 2021;

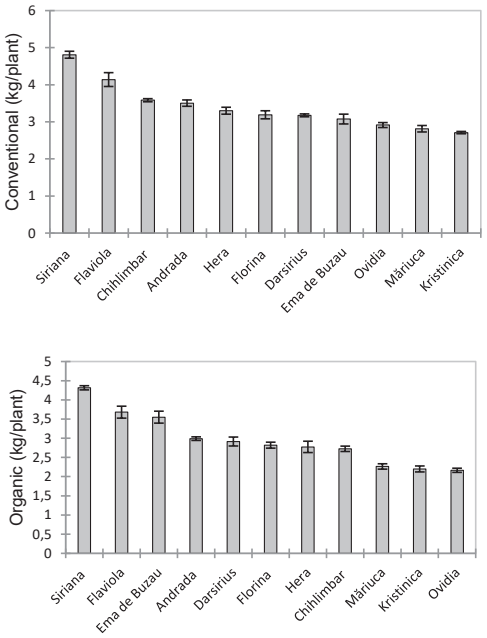


Figure 1. Tomato yield in both farming systems

For peppers (Figure 2), A-50 achieved the highest conventional yield, while Regal excelled in organic farming. Hot pepper cultivars (Roial and Decebal) had the lowest organic yields. Differences in yield are influenced by genotypic tolerance (Udriște et al., 2024) to environmental stresses and soil nutrient availability, as noted in previous research on *Capsicum* spp. (Hallmann & Rembiałkowska, 2012).

Eggplant results (Figure 2) showed Rebeca performing best in both systems, while specialty cultivars Iarina (slim-green) and Romanița (white) recorded the lowest yields. Specialty eggplants often prioritize aesthetic and culinary traits over productivity, which may explain their lower yield performance (Kumar et al., 2020).

Tomato yields in organic farming were comparable to conventional systems, suggesting that some genotypes perform well under low-input conditions. However, multi-season trials are needed to confirm long-term stability (Bàrberi, 2015).

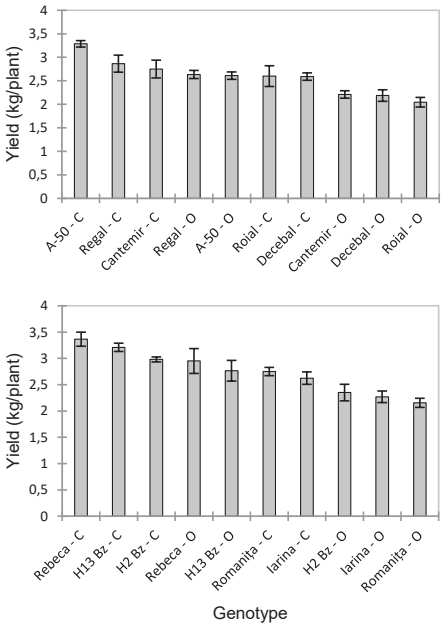


Figure 2. Pepper and aubergine yield in both farming systems

Peppers showed greater system-dependent variation, with higher yields in conventional systems but enhanced antioxidant content in organic farming (Scalzo et al., 2005; Soare et al., 2017).

Eggplants demonstrated strong adaptability, performing well in both systems. This resilience suggests that eggplants could be a suitable crop for organic expansion, given their natural resistance to biotic stress (Kumar et al., 2020).

Physicochemical properties of Solanaceous fruits

Tomato quality assessments (Table 1) revealed significant cultivar-based differences in firmness, dry matter (TDM), betacarotene and lycopene content. The highest firmness (7.18 N) was observed in Florina O, while Ema de

Buzău C had the softest fruits (1.18 N). Firmness is a key quality attribute influencing storage and transportability, and these values align with previous reports showing that processing tomatoes tend to have firmer textures than fresh-market varieties (Tigist et al., 2011; Flores-Velasquez et al., 2022).

The TDM content varied significantly, with Ema de Buzău O showing the highest (10.81%) and Florina O the lowest (4.72%). Dry matter content is directly related to sugar and organic acid concentrations, influencing taste and nutritional quality (Beckles, 2012; Dinu et al., 2023). Organic tomatoes often have higher dry matter content due to slower growth rates and reduced nitrogen availability (Zhao et al., 2012).

Tabel 1. Comparative physicochemical properties of tomatoes and peppers in conventional and organic farming

Genotypes	Firmness (N)	TSS (% Brix)	TDM %	FW (g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)
A-50 M - O	8.053	6.003	9.647	168.900	6.633	8.803
A-50 M - C	8.231	5.941	9.750	171.860	5.931	8.311
Roial M - O	6.860	8.390	14.580	24.990	3.630	7.330
Roial M - C	7.030	9.050	14.190	26.720	3.130	6.930
Chihlimbar O	5.445	5.425	6.905	191.710	0.015	16.115
Ema de Buzau O	1.180	6.140	10.810	9.880	8.310	9.310
Decebal M – O	7.900	8.160	11.670	33.130	0.010	4.810
Chihlimbar C	5.560	5.100	6.960	211.330	0.010	15.210
Ema de Buzau C	1.225	6.085	10.015	10.750	6.215	8.815
Regal M - O	8.880	5.950	4.110	140.160	3.820	6.220
Decebal M - C	7.941	7.981	11.980	38.980	0.011	4.111
Andrada C	4.571	4.881	6.450	290.950	9.221	3.611
Andrada O	4.448	5.048	6.333	249.310	12.718	3.718
Cantemir M – O	10.002	3.842	7.808	118.600	5.202	4.802
Regal M - C	8.952	5.822	4.249	142.180	3.312	5.812
Cantemir M - C	10.562	3.722	7.929	121.450	4.802	4.302
Florina O	7.192	4.392	4.728	171.880	7.312	4.712
Florina C	7.183	4.423	4.867	200.690	5.213	4.513
Hera O	2.981	5.391	7.470	153.390	5.511	3.811
Mariuca O	5.765	4.695	7.635	85.630	5.515	3.815
Hera C	3.156	5.156	7.584	169.350	4.106	3.406
Darsirius C	5.672	4.852	6.788	121.110	6.902	2.902
Darsirius O	5.625	4.835	6.615	111.770	7.525	3.325
Kristinica O	3.702	4.542	6.699	128.580	5.702	4.702
Mariuca C	5.850	4.740	7.700	100.750	4.320	3.120
Flaviola O	2.792	7.992	6.669	27.860	4.012	5.022
Kristinica C	3.761	4.631	6.849	138.560	5.201	4.101
Flaviola C	2.781	6.231	6.780	32.380	3.241	4.511
Siriana O	3.925	4.435	4.945	131.060	3.505	3.205
Siriana C	4.000	4.400	4.980	158.870	2.910	2.910
Ovidia O	1.313	4.513	7.237	56.470	0.013	1.713
Ovidia C	1.471	4.121	7.310	89.570	0.011	1.211

Dry matter and sugar content play crucial roles in flavour development and consumer acceptance. Ema de Buzău had the highest TDM, supporting previous research that smaller fruits often have higher dry matter concentrations (Beckles, 2012).

Lycopene content was highest in Andrada O (12.71 mg/mL), while Chihlimbar O exhibited

the highest β-carotene levels (16.11 mg/mL). Ovidia lacked lycopene, likely due to genetic mutations affecting carotenoid biosynthesis (Pankratov et al., 2016; Yoo et al., 2017).

These findings are consistent with studies showing that organic tomatoes tend to accumulate higher antioxidant compounds due to enhanced stress responses (Baranski et al., 2014).

Lycopene and β-carotene content were highly genotype-dependent, with yellow and orange cultivars lacking lycopene due to genetic mutations (Agarwal & Rao, 2000). Organic farming may enhance carotenoid accumulation due to increased oxidative stress responses (Baranski et al., 2014).

Pepper firmness varied by cultivar and maturity stage (Table 1), with Cantemir C showing the highest firmness (10.56 N). Firmness is a critical indicator of postharvest quality and consumer preference, as noted by Palacio and Sánchez (2017).

Dry matter content was highest in Roial C (14.58%) and lowest in Regal C (4.24%). Higher dry matter values indicate better storage potential and richer flavour (Conforti et al., 2007).

Lycopene was detected only in red fruits, with A-50 O containing the highest level (6.63 mg/100g). Previous research confirms that lycopene biosynthesis increases with ripening and is influenced by genotype and growing conditions (Hallmann & Rembiałkowska, 2012). β-carotene content was highest in red peppers, reinforcing findings that carotenoid accumulation is strongly correlated with fruit maturity and light exposure (Kurina et al., 2021; Lanoue et al., 2024).

Eggplant firmness and dry matter content varied significantly, with Rebeca, H13Bz, and H2Bz showing superior firmness, while Iarina and Romanița had softer textures. Firmness is an essential parameter for marketability, as firmer fruits have longer shelf lives (Kumar et al., 2020).

Dry matter ranged from 5.16% (Romanița) to 10.12% (Rebeca). Higher dry matter is linked to enhanced flavour and nutritional density, particularly in organic crops where stress conditions promote secondary metabolite accumulation (Alsina et al., 2021).

Overall, these results highlight the need for tailored genotype selection for organic farming.

CONCLUSIONS

The study highlights the potential of specific Solanaceous cultivars for organic farming, addressing both yield stability and fruit quality. Darsirius tomato and Regal pepper demonstrated consistent productivity across both organic and conventional systems, while Rebeca eggplant exhibited strong resistance to pests and diseases. Nutritional quality varied significantly among cultivars, with Ema de Buzău showing the highest dry matter content and Andrada having the highest lycopene concentration. These findings support the selection of robust, adaptable cultivars for sustainable organic agriculture. Future research should focus on multi-season trials and breeding efforts to optimize crop performance under organic conditions.

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THE PERSPECTIVES OF HYDROPONICS CULTIVATION SYSTEM: A REVIEW

Mihaela Carmina BENCHEA, Dan Ioan AVASILOAIEI, Mariana CALARA, Claudia BĂLĂIȚĂ, Creola BREZEANU

Vegetable Research and Development Station Bacău, 220 Calea Bârladului, Bacău, Romania

Corresponding author email: avasiloaiei_dan_ioan@yahoo.com

Abstract

Hydroponics is a growing technique that uses aqueous media (nutrient solution) to support plant growth. This technology offers viable solutions to today's global challenges. In the context of the decreasing and degrading of available agricultural land, hydroponic systems can contribute to food security. The main objective of this study is to analyze the current hydroponic techniques and nutrient solution management. Additionally, the study explores the groundbreaking advancements in hydroponic cultivation, emphasizing the integration of sensor-based monitoring systems and automated control technologies, which collectively enhance resource efficiency and optimize plant growth conditions while enabling precise, data-driven agricultural practices. The conclusions of the study emphasize that hydroponic agriculture is an alternative with high potential for increasing crop yields and optimizing the use of limited resources.

Key words*: growing technique, nutrient solution, food security, smart farming.*

INTRODUCTION

The amount of land and water available for agricultural crops has decreased throughout centuries due to urbanization, population increase and soil degradation (Dobermann et al., 2013). According to estimates, half of the world's land will not be adequate for agriculture by 2050 (Olowe, 2021). The United Nations (UN) claims that there is a food crisis in several nations right now (Okemwa, 2015). As a result, contemporary methods for attaining sustainable agriculture have begun to attract interest (AlShrouf, 2017).

Climate change, more frequent floods and droughts, soil erosion and degradation from traditional farming practices that deplete the soil of vitamins and minerals are the main causes of this collapse. As a result, methods to enhance and boost agricultural systems' production must be developed and implemented globally (Gashgari et al., 2018).

Because there are a number of negative environmental repercussions associated with traditional agriculture methods, growing techniques that are soilless have gained popularity (Killebrew & Wolff, 2010; Walls, 2006).

The earliest published work on growing land plants without soil was found in the book *"Sylva Sylvarum"* by Francis Bacon in 1627 (Swain, 2021). The term "Soilless culture" mostly refers to hydroponic, aeroponic and aquaponic methods.

The term hydroponics was derived from the Greek words "hydro", meaning water and "ponos", meaning labor (Biebel, 1958).

The earliest records of hydroponics in use were in the Hanging Gardens of Babylon, where plants were grown in a continuous stream of water (Wahome, 2015).

Dr. Gericke, a professor from California, introduced the term "hydroponics" in 1929 as he started to transform a previously lab-based method into a commercial way of cultivating plants (Sardare & Admane, 2013). The phrase was still in use until the 1980s, although it became more inclusive (Ciofu, 2003).

According to some authors, hydroponics is a technique of growing plants in nutrient solutions (containing water with fertilizers) with or without the use of an inert medium (substrate: sand, gravel, vermiculite, rockwool, perlite, coconut fiber) to provide mechanical support (Sharma et al., 2018). In 1983, Collin and Jensen expanded the definition of "hydroponics" to encompass the entire spectrum of crops cultivated on both organic and mineral substrates (Ciofu, 2003; Draghici & Jerca, 2021).

Hydroponics is an excellent way for people who don't own land to grow plants as a hobby because it can be done in extremely small spaces, with little work and little time (Barman, 2016).

During World War II, the US military employed use hydroponics to produce fresh food for soldiers positioned on desolate Pacific islands. By the 1950s, there were successful commercial farms in Asia, Africa, Europe and the Americas (Sardare & Admane, 2013).

In Romania, the first experiments in the field of hydroponic crops on nutritive solutions were carried out by the researchers of the vegetable-growing section of the former Horticultural Research Institute in the 1960. In the late 1980s, at the Faculty of Horticulture in Cluj, an experimental program was started to develop unconventional technologies for the production of tomatoes and cucumbers in greenhouses. Also during that period, at the Bucharest-Berceni Greenhouse Complex, an area of 1 ha was set up and exploited for 2 years for the cultivation of tomatoes on mineral wool, fed with nutrient solution in an open circuit (Ciofu et al., 2003).

MATERIALS AND METHODS

This study primarily focuses on a comprehensive analysis of the resources accessible within the global body of scientific literature.

The Google Academic, Free Full PDF, Research Gate, Science Direct, MPDI, Frontiers, Web of Science, PubMed, and others easily accessible online resources provided the data for this investigation. To identify the content of our work, we searched for phrases and terms like “soilless cultures”, “hydroponic growing techniques”, “nutrient solutions” and “modern technics in hydroponics”.

This document comprises exclusively credible research articles published in peer-reviewed scientific journals in recent years, presenting established advancements and future prospects for hydroponic growth systems.

In accordance with these standards, 114 studies were incorporated into the analysis, influencing the final amendment. Only studies that satisfied predefined criteria were considered.

RESULTS AND DISCUSSIONS

Vegetables including tomatoes, lettuce greens, cucumbers and peppers, as well as decorative crops like herbs, roses, freesias and foliage plants, can be produced using hydroponics (Sardare & Admane, 2013).

The main crops suitable for hydroponic cultivation are systematically presented in Table 1.

Table 1. Crops suitable for hydroponically growing

Type of culture	**Crop name**
Cereals	*Oryza sativa* (rice), *Zea mays* (corn)
Fruits	*Fragaria ananassa* (strowberies)
Vegetables	*Lycopersicon esculentum* (tomatoes), *Capsicum frutescens* (chili pepers), *Solanum melongena* (eggplant), *Phaseolus vulgaris* (green bean), *Beta vulgaris* (sugar beet), *Psophocarpus tetragonolobus* (peas asparagus), *Capsicum annum* (pepper), *Brassica oleracea var. capitata* (cabbage), *Brassica oleracea* var. *botrytis* (cauliflower), *Cucumis sativus* (cucumber), *Cucumis melo* (melon), *Raphanus sativus* (wrinkles), *Allium cepa* (onion)
Leafy vegetables	*Lactuca sativa* (salad), *Ipomoea aquatica* (spinach)
Spices	*Petroselinum crispum* (parsley), *Mentha spicata* (ment), *Ocimum basilicum* (basil), *Origanum vulgare* (oregano)
Ornamental flowers-crops	*Tagetes patula* (tagetes), *Rosa berberifolia* (roses), *Dianthus caryophyllus* (carnation), *Chrysanthemum indicum* (chrysanthemum)
Medicinal crops	*Aloe vera* (aloe), *Solenostemon scutellarioides* (Coleus)
Forage crops	*Sorghum bicolor* (sorghum), *Medicago sativa* (lucerne), *Hordeum vulgare* (barley), *Cynodon dactylon* (pir gros), *Axonopus compressus* (Carpet grass)

In contemporary agriculture, hydroponic cultivation represents one of the most advanced and intensive crop production methodologies (Swain, 2021). These systems are a viable agricultural alternative for non-fertile or metal-contaminated soil (Supraja et al., 2020) and have a quicker growth rate (30-50%) Huo, 2020.

Hydroponic technology allows optimal utilization of nutrient solutions, water and space, as well as better control of climate and plant

protective factors. Hydroponic technology can be an effective means for food production in ecosystems and extreme environments, such as deserts, mountainous regions, or arctic communities (Gaikwad & Maitra, 2020). For instance, people living in underdeveloped and poor regions of Thailand cannot grow enough food using traditional agricultural practices due to high soil salinity and lack of natural nutrients in the soil, but hydroponic systems can successfully generate additional crop yields (Ortiz et al., 2009).

Growing in popularity around the world, hydroponic systems provide growers and consumers with a variety of new chances to produce high-quality harvests, such as vegetables improved with bioactive substances (Jan et al., 2020).

Europe is recognized as the predominant hub of the hydroponics market, with France, the Netherlands, and Spain emerging as the leading producers, followed by the United States and the Asia-Pacific region (Prakash et al., 2020).

Hasan et al. (2018) stated that hydroponic systems frequently necessitate specialized infrastructures and a controlled microclimate, which can be regulated within protected cultivation environments.

Hydroponic crop classification is primarily determined by factors such as drainage systems, substrate and container types, as well as the nutrient delivery mechanisms employed (Hasan, 2018).

There are a number of different systems and techniques used that fall under the category of 'hydroponics'. According to some authors, hydroponic techniques have been divided into two categories, as presented in Table 2: growing in solution and growing in solid media (growing media) (Atherton & Li, 2023).

Tabel 2. Classification of hydroponic tehniques

Culture in nutrient solution		**Culture in solid media**
Non-circulating systems (open system)	**Circulating systems (closed system)**	
Root-sinking technique	Nutrient film technique (NFT)	Hanging bag technique
Floating technique	Deep flow technique (DFT)	Grow bag technique
Capillary action technique	Drip technique	Pot technique
	Wick technique	Trench/trough technique
	Flood and flow technique	

1. Solution culture: plant roots are placed directly in liquid (nutrient solution). Solution culture can be subdivided into circulating and non-circulating systems (Atherton & Li, 2023).

A. Non-circulating systems (open system) in which excess nutrient solution not utilized by plants is discharged to the environment (Ciofu et al., 2003).

- *Root submersion technique*: plants are suspended above the nutrient solution, with their roots partially immersed in the solution to a depth of approximately 2-3 cm (Atherton & Li, 2023).

- *Floating technique:* the plants are affixed to a lightweight polystyrene plate, which floats atop the nutrient solution, ensuring that the roots remain fully submerged within the solution (Hussain et al., 2014).

- *Capillary action technique:* pots filled with an inert medium feature perforations at the base and are positioned in shallow containers that hold the nutrient solution (Hussain et al., 2014). The nutrient solution reaches the plant roots by capillary action. The technique is suitable for ornamentals, flowers and indoor plants (Swain et al., 2021).

B. Circulating systems (closed system): in which the nutrient solution is collected, reconditioned and recirculated to the growing facilities (Ciofu et al., 2003; Draghici & Jerca, 2021).

- *Nutrient Film Technique* (NFT): NFT was developed in the mid-1960s in England by Dr. Alen Cooper to overcome the shortcomings of ebb and flow systems. The nutrient film technique (NFT) is the most popular hydroponic system. In this method, the plants are placed in a polyethylene tube that has slits cut for the roots to be inserted (Shrestha & Dunn, 2010). The nutrient solution is constantly pumped through the tube in which the plants are placed (Ciofu et al., 2003).

- *Deep flow culture* (DWC): This method involves suspending plant roots in nutrient-rich water. Because algae and molds can grow quickly in the reservoir, it is essential to closely monitor the oxygen and nutrient concentrations, pH and salinity. This approach can be used to cultivate vegetables like tomatoes and cucumbers with great success (Swain et al., 2021).

- *Drip technique:* a drip emitter is used to periodically supply the nutrient solution to a substrate that has plant roots on it (Fussy & Papenbrock, 2022). By using nozzles, the drip emitter slowly disperses nutrients and extra solutions can be gathered and recycled or simply let run off. With this technique it is feasible to cultivate multiple plant species at the same time (Jan et al., 2020).
- *The wick technique*: is the simpliest hydroponic system that doesn't need aerators, pumps or power (Swain et al., 2021). Plants are submerged in an absorbent medium, such as perlite, vermiculite, etc., that transfers from the roots of the plants into a nutrient solution reservoir. Capillary action is how plants get their nourishment (Fussy & Papenbrock, 2022). Small plants, herbs and condiments respond well to this approach (Jan et al., 2020).
- *Flood and drain technique:* this is the first commercial hydroponic system. This system uses a growing tray and a reservoir that is filled with a nutrient solution. A pump periodically floods the growing tray with nutrient solution, which is then slowly drained. It is possible to grow different types of plants, but careful attention must be paid to the problem of root rot, algae and mold; therefore, a system with modified filtration is required (Jan et al., 2020).

2. Development on solid medium (in growth media): This approach contrasts with direct solution-based cultivation, as the plants are fully supported by a solid substrate. Fertigation is employed to deliver the nutrient solution either on top of or beneath the substrate surface (Atherton & Li, 2023).

Numerous research studies have been conducted in the research greenhouse of the Research Center for the Study of Agro-Food Product Quality within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, from 2014 to the present. These studies have focused on optimizing tomato cultivation technologies on nutrient substrates, with particular emphasis on the use of perlite as a growing medium (Drăghici et al., 2021; Stoica et al., 2021; Stoica et al., 2022).

- *Hanging bag technique:* this method entails suspending polyethylene bags, approximately one meter in length and filled with a culture medium, over a gutter or channel. Plants are placed in mesh pots positioned within holes cut into the sides of the bags. The nutrient solution is pumped from a reservoir to the top of the bags, where it percolates through the culture medium, drains into the gutter, and is subsequently recirculated back into the reservoir (Waiba et al., 2020).
- *Grow bag technique:* growth media-filled polyethylene bags are set on the ground. Seedlings or seeds are inserted into the bag after tiny holes have been made in it. After that, each plant receives a nutrient solution via pipes (Sengupta & Banerjee, 2012).
- *Potting technique:* is similar to the growing bag technique, except that plants are placed in individual pots filled with culture medium and individually fertigated (Hasan et al., 2018).
- *Trench/trough technique:* trenches or troughs created above the soil are lined with an impermeable material and filled with inert medium. Plants are then placed in the trough at intervals and are supplied with nutrient solution by drip irrigation (Fussy & Papenbrock, 2022). Sometimes, a drainage pipe is placed at the bottom to allow excess solution to drain into the soil (Waiba et al., 2020).

Management of nutrient solutions used in hydroponics

When discussing hydroponic growth systems, the nutrient solution is the most important determinant of crop quality (Asao, 2012). For hydroponic crops, nutrient solution is a significant and necessary supply of nutrients (Patil et al., 2020).

According to Steiner (1961), the nutrient solution utilized in hydroponic systems is an aqueous solution mostly composed of soluble salts of inorganic ions of the elements necessary for plants. Iron chelates and other chemical compounds can occasionally be found as well (Suazo-López et al., 2014).

Trejo-Téllez et al. (2007) noted that elements such as sodium, silicon, vanadium, selenium, cobalt, aluminium, and iodine are regarded as beneficial due to their ability to stimulate plant growth. These elements may mitigate the toxic effects of other substances or serve as substitutes for essential nutrients in a less specific capacity. Nutrient solutions, which form the foundation of hydroponic systems, primarily consist of macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur),

with additional supplementation of micronutrients (Patil et al., 2020).
Electronic conductivity (EC), pH, concentration, temperature and nutrient solution formula are the main determinants of nutrient solution quality (Patil et al., 2020).

a. ***Nutrient solutions formula***
Proper nutrient solution formation and management are the keys to success for hydroponic crop production (Patil et al., 2020).
To facilitate the use of nutrient solution, there are a number of formulations available on the market to choose from. However, hydroponic growers can also prepare their own fertilizer mixtures to prepare nutrient solutions using fully water soluble nutrients. When preparing a nutrient solution, it is crucial to select fertilizers that are chemically compatible, as certain fertilizer salts may react adversely with one another. For instance, ammonium sulfate combined with potassium chloride results in the formation of less soluble potassium sulfate in the tank. Similarly, phosphate fertilizers can cause issues when present in high concentrations of calcium and magnesium, leading to the precipitation of poorly soluble phosphates (Kumari et al., 2018). Kong and Iersel (2004) reported significant increases in shoot growth, total dry weight, and leaf area in sage crops as the nutrient solution concentration was elevated from 0.125 to 1.0.
Nada et al. (2010) suggested that the critical boron concentration in nutrient solution is 4 ppm for long-term hydroponic tomato cultivation
De Bever et al. (2013) observed that the photosynthetic rate, evapotranspiration and intercellular CO2 concentration and chlorophyll content of *Beta vulgaris* (sugar beet) were generally higher in nutrient solution treated with nitrogen that was readily available to plants
Lopez-Pozos et al. (2011) found that inadequate oxygenation of nutrient solution (NS) in recirculated hydroponic systems leads to root hypoxia as a result of low oxygen solubility. Hypoxia affects crop nutrients and water uptake and results in reduced crop yield.

b. ***Electrical conductivity (EC) and pH of the nutrient solution***
It has been demonstrated that a culture can yield a diverse array of species by regulating the concentration of the nutrient solution, commonly referred to as electrical conductivity or osmotic pressure (Libya et al., 2012; Trejo-Téllez & Gómez-Merino, 2012; Kang & Van Iersel, 2009).
Sonneveld & Voogt (2009) reported that nutrient composition determines the electrical conductivity and osmotic potential of the solution. The ideal electrical conductivity (EC) is crop specific and dependent on the environmental conditions; however, values of electrical conductivity (EC) for hydroponic systems range from 1.5 to 2.5 ds m^{-1} (Sonneveld et al., 2009).
Samarakoon et al. (2006) found that higher electrical conductivity (EC) hinders nutrient uptake by increasing osmotic pressure, while lower electrical conductivity (EC) can severely affect plant health and yield (Samarakoon et al., 2006).
Low electrical conductivity implies a low nutrient concentration, which usually leads to nutrient deficiencies and slow plant growth rates; however, very high levels can burn and/or kill plants. Cuttings and tender plant seedlings can experience burns if the conductivity/ TDS is too high. Only after the plant begins to grow does it need a stronger nutrient solution. Some plants prefer milder nutrient strength, while other plants produce better-quality fruit with higher nutrient strength (Gaikwad & Maitra, 2020). Increased electrical conductivity in the nutrient solution tends to restrict the movement of calcium to developing fruit and increases the incidence of blossom end rot (BER), a physiological disorder that is associated with low calcium concentrations, especially at the distal end of the fruit (Adams & Ho, 1989).
When the nutrient solution is recycled, ions can accumulate, resulting in a significant increase in electrical conductivity (EC). This accumulation causes nutrient imbalances (depletion of some nutrients), affecting both yield and quality of the crop. The high concentration of sulfate in the nutrient solution reduces foliar Ca concentration and relative calcium activity but does not affect fruit yield or foliar content (Lopez et al., 2002).
Rafaela et al. (2017) revealed that the yield of the lettuce crop reduced considerably with increasing electrical conductivity and eventually osmotic pressure. The stress of saline exposure fundamentally interferes with plant functions such as photosynthesis and protein synthesis.

Saline stress inhibits plant growth by osmotic effect, restricting water availability and disturbing nutrition (Guimaraes et al., 2017).
The pH of the nutrient solution plays an important role in hydroponic cultivation. When the pH is not at the appropriate level, plants will lose their ability to absorb some of the essential elements needed for healthy growth. For all plants, there is a certain optimal pH level (Trejo-Téllez & Gómez-Merino, 2012). This pH level will vary from plant to plant; the recommended pH for hydroponic agriculture is between 5.5 and 6.5. This is because the overall availability of nutrients is optimized at a slightly acidic pH (Patil et al., 2020).
Ahn and Ikada (2004) found that the optimal pH is between 5 and 7, and the optimal concentration of the nutrient solution is ¼ to 1 unit of standard solution (Ahn & Ikeda, 2004).

Contamination control in hydroponic systems

In hydroponic cultures, maintaining a sterile environment in the root zone is essential for good plant vigor. The application of pesticides is generally avoided in hydroponic systems. With reduced pest problems and constant feeding of the roots with nutrients, productivity in hydroponics is high, despite limited plant growth due to low atmospheric carbon dioxide levels or limited light (Sardare & Admane, 2013).
However, although hydroponic cultivation is much easier to control in terms of contamination, this type of cultivation can still suffer. A disease commonly encountered in hydroponic cultivation is wilting, caused by *Fusarium* and *Verticillium* (Raviv et al., 1998). Only the thermal treatment of the nutrient solution was found effective in keeping the root zone free of pathogens (Savvas, 2002).

The advantages and disadvantages of hydroponics

There have been many questions regarding whether hydroponic crops are actually more efficient than soil-based crops. An experimental study conducted by Maeva Makendi showed a competitive analysis between growing plants hydroponically and growing plants in soil systems. The experiment was conducted on different types of plants over the course of a month. The hypothesis stated: "If hydroponic plants and soil-grown plants are given the same germination and growth conditions, then hydroponic plants will perform just as well, if not better, than soil-grown plants". It was concluded that hydroponic plants germinated and grew faster than soil-grown plants (Montgomery, 2017).
Thus, a comprehensive comparison between hydroponic systems and soil-based cultivation systems reveals numerous advantages (Savvas, 2002); however, soilless culture also presents certain limitations (Sonneveld, 2000). A thorough SWOT analysis of these systems, outlining both their strengths and weaknesses, is presented in Table 3.

Table 3. SWOT analysis of hydroponic system

Swat analysis		
Key Components	**Description**	**Literature**
Strengths	Hydroponic systems produce the healthiest crops with high yields and are consistently reliable.	Silberbush & Ben-Asher, 2001
	Hydroponic system requires very little work and is very simple and clean.	Silberbush & Ben-Asher, 2001
	Considering that nutrients are delivered straight to the roots in hydroponic systems, plants develop more quickly and have fewer roots.	Silberbush & Ben-Asher, 2001
	Space is saved as only 1/5 of the total space is used.	Silberbush & Ben-Asher, 2001
	Since there is little chance of weed emergence, chemical treatments are not necessary.	Sonneveld, 2000
	Because hydroponic cultivation guarantees effective nutrient management, production per acre increases and product quality improves.	Sonneveld, 2000
	Work for various intercultural operations such as plowing, cultivating, watering and other practices is largely eliminated.	Swain et al., 2021

	This technique can use fertilizers and water effectively. Consequently, there is little possibility of losing valuable chemicals; it may result in less land pollution.	Swain et al., 2021
	The plants are grown locally, therefore the excess carbon can be reduced.	Swain et al., 2021
	Hydroponic growth also reduces soil problems, such as seed degradation, seedlings, and diseases related to soil-dwelling fungi.	Geilfus & Geilfus, 2019
Weaknesses	The application on a commercial scale requires technical knowledge and a large initial investment.	Sonneveld, 2000
	Hydroponics is limited to high-value crops given the high cost.	Swain et al., 2021
	Is needed a strong and unlimited source of electrical energy for the system.	Van Os et al., 2002
	Before starting the cultivation of any vegetable crop, proper training is an important prerequisite.	Swain et al., 2021
	It is very important to understand how plants grow and the principle of nutrition.	Swain et al., 2021
	The grower must observe the plants every day, as the plant's reaction to good or deficient nutrition is incredibly rapid.	Swain et al., 2021
	Cultivators must have knowledge of climate control within the structure.	Swain et al., 2021
	Crops grown in soil benefit from the mineralization of nutrients in the soil and microbial activity, whereas hydroponic plants rely exclusively on the addition of fertilizer to the nutrient solution.	Djidonou & Leskovar, 2019
	These systems have the disadvantage of requiring large inputs of water and fertilizers.	Kempen et al., 2017
Opportunities	In areas of the world where there is a shortage of arable or fertile land for agriculture, it works efficiently.	Sonneveld, 2000
	The maximum yield can be obtained by making the system economically feasible on high-density and expensive land.	Swain et al., 2021
	It is possible to produce vegetables 'out of season', thus providing an economic benefit to growers.	Swain et al., 2021
Threats	There is a chance of rapid spread of substrate-borne diseases and nematodes to all beds on the same nutrient reservoir of a closed system.	Swain et al., 2021

Smart hydroponic

Due to the fact that hydroponics creates an artificial growth environment, it requires automation technology to maintain and keep it within the correct range (Jain and Kaur, 2024). Artificial intelligence-based systems and Internet of Things (IoT) automated growing procedures are two examples of innovative technologies that have lately gained popularity and have practical applications in indoor hydroponic operations (Javaid et al., 2020; Rajaseger et al., 2023). Smart agriculture relies on communication technologies and information for decision-making (Abd Ghani et al., 2023). Industry 4.0 and the application of technologies such as IoT, cloud computing, AI and ML (machine learning) to predict growth parameters for different plants (Mamledesai et al., 2020) can make hydroponics even more efficient (Ragaveena et al., 2021).

Each plant requires a varied set of nutrients because they have varying pH and EC ranges for different growth phases. It is difficult for farmers to keep track of these and manually control everything (Shetty et al., 2021). As technology advances, the integration of smart technologies, automation and data analysis in hydroponics further enhances its potential. This integration allows for real-time monitoring, precise control and automation of various processes, leading to greater efficiency, reduced labor requirements and improved overall productivity (Resh, 2022).

IoT-assisted parameter control

In recent years, numerous efforts have been made to develop smart agricultural systems capable of monitoring and controlling various plant growth parameters, such as light intensity, temperature, resource usage, energy consumption, relative humidity, electrical

conductivity, dissolved oxygen and pH (Gillani et al., 2022). The problem with hydroponic agriculture is the continuous requirement to maintain and control the artificial growth environment to allow optimal plant growth. The quality of the plants grown on the farm can be significantly affected by changes in climate, natural light and fertilizer solution at any time during the plant's growth cycle. According to studies, each increase of 3°C in the ambient temperature within the optimal range reduces crop productivity by 10% to 40%. Therefore, by automatically providing the ideal growth conditions throughout the growth cycle, IoT-based automation is crucial for achieving optimal plant growth (Jain and Kaur, 2024).

IoT can be defined as a vast network that allows the connection of any objects and people anytime and anywhere, using a service (Perera et al., 2014; Jin et al., 2014). In recent years, the number of devices encompassing the Internet of Things (IoT) has increased from 0.9 billion objects in 2008 to 50 billion by the end of 2020, making it a part of every existing application (Perera et al., 2014; Ghobaei-Arani et al., 2019; Kour et al., 2021; Chauhan et al., 2021). Sensors are the backbone of IoT networks and are an integral part of all IoT applications, including precision agriculture (Shieh et al., 2001; Antonacci et al., 2018). They are also used in various smart agriculture solutions, including hydroponics (Joshitha et al., 2021). The sensor network used in IoT can continuously monitor and control the health and growth of crops. All essential macro- and micronutrients required for crops are delivered to the plant through channels or other media (Trejo-Téllez et al., 2012). Agronomic growth variables can be both monitored and controlled by farmers through application interfaces directly connected to the cloud that stores real-time data (Palande et al., 2018).

Using IoT and automation techniques, a digital twin for hydroponics was developed that measures parameters such as pH, EC, water humidity and temperature in real-time and also predicts the growth and fresh weight of crops (Reyes et al., 2022). Boonnam et al. (2017) designed a hydroponic system using IoT and a wireless sensor network (WSN). Different important parameters, such as light, humidity and temperature, were controlled using this system. The data obtained from the sensors was continuously sent to the cloud for storage and further use. The parameters were controlled using a mobile application.

Recently, there have been reports about a smartphone application developed in Android Studio that allows users to adjust and monitor plant growth in hydroponic vertical farming systems. Using Internet of Things (IoT) technology, sensors are used to monitor environmental and dietary factors, including temperature, humidity, TDS, pH and water level. The Thing-Speak cloud platform was then used to send the data. The Tashi Home Pindfresh system and Arduino and Raspberry Pi were used as control centers (Kaur et al., 2022).

Despite these advantages, the monitoring and control of IoT parameters face challenges such as the lack of standards, high initial costs and security issues caused by network encryption (Gillani et al., 2022).

AI and ML for parameter optimization

Artificial intelligence (AI) and machine learning (ML), combined with big data and IoT, are driving the digitalization of agriculture. These technologies have the potential to improve real-time monitoring, increase production and achieve greater control over crop growth (Talaviya et al., 2020). ML algorithms and techniques are implemented in various aspects of agriculture, including crop management (yield prediction, species recognition, disease detection), and water and soil management (type, pH, temperature, moisture content) (Liakos et al., 2018).

A hydroponic system based on multimodal data analysis has demonstrated the ability to monitor and optimize six growth parameters (ambient temperature, airflow speed, relative humidity, CO2 concentration, pH, EC and nutrient solution temperature) related to plant growth in a hydroponic plant factory (Lam et al., 2020).

A group of researchers has developed a control kit applicable to any hydroponic system of any size to control some of the growth parameters (pH, EC, nutrient solution temperature) at an optimal level. This kit was able to increase lettuce production by 21% compared to uncontrolled growth (Chuah et al., 2019).

A test bed based on an open IoT platform has been developed for monitoring hydroponic systems. It optimizes and monitors parameters,

sends alerts if the parameters are not within optimal limits and estimates energy consumption. This testing platform provides essential information regarding the optimization and correlation of various growth parameters (Chowdhury et al., 2020).

Control of parameters in hydroponics 4.0/5.0

Hydroponics offers a solution to food insecurity, enhances sustainability and allows the integration of modern technologies (Shareef et al., 2024).

In 2021, the European Commission officially adopted Industry 5.0, which emphasizes sustainable, resilient and human-centric production systems and technology (Xu et al., 2021; Maddikunta et al., 2022). The application of Industry 5.0 principles in hydroponics, referred to as Hydroponics 5.0, can significantly improve agricultural production and its quality. For hydroponic systems, a cyber-physical cognitive system (CPCS) can be developed, capable of perceiving situations and taking corrective actions accordingly. This involves assigning repetitive tasks, such as monitoring parameters, controlling and optimizing subsequent actions to cobots (collaborative robots), while humans handle critical thinking. Digital twins can help detect diseases and predict yield/growth, leading to better control during the growth period. This approach paves the way for sustainable, resilient, and climate-smart hydroponic systems (Abbasi et al., 2022; Xu et al., 2021; Maddikunta et al., 2022).

CONCLUSIONS

The quality and quantity of crops produced, as well as the effectiveness of the growing environment and environmental preservation, are all greatly enhanced by the hydroponics techniques approach. The main benefit is that, in comparison to traditional agriculture, water usage can be reduced by up to 90% because the system is closed and the water is recirculated.

This growing method is perfect for cramped areas and urban settings since it saves space and makes effective use of light. Growers can transform their terraces and balconies into fruitful little gardens so they can unwind while caring for their own crops and enjoying the results of their labor.

When we talk about hidroponics we talk about a wide range of techniques that allow better control of climate and plant protection factors making it a promising agricultural alternative for the future.

Is a method that increases the quality and productivity of crops by avoiding the use of pesticides while having reduced problems with diseases and pest pests.

The most difficult issue with hydroponic agriculture is the constant need to maintain and regulate the artificial growing environment to allow for optimal plant growth. This type of crop is easily adaptable and enables the integration of contemporary agricultural technologies to automatically provide ideal growing conditions (with the help of sensors regulating pH, humidity, temperature, etc.) throughout the cycle.

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CRAFTING UNIQUE BLENDS: EXPLORING COMBINATIONS OF ONION, GARLIC, WILD GARLIC AND LEEK

Viorica BOACĂ, Despina-Maria BORDEAN, Codruța GAVRILĂ, Laura RĂDULESCU, Alexandra BAN-CUCERZAN, Narcis Gheorghe BĂGHINĂ

University of Life Sciences "King Mihai I" from Timișoara,
119 Calea Aradului, Timișoara, Romania

Corresponding author email: despinabordean@usvt.ro

Abstract

Due to their high content of phytonutrients, Allium plants are used both as food and in the treatment of many medical conditions. The increased interest in these plants is due to their powerful antioxidant properties. In this paper, the Barchart logarithmic diagram allowed us to identify the representative nutritional components for each investigated product (onion, garlic, wild garlic and leek plants), allowing us, at the same time, to identify potential mixtures of dehydrated powders to be recommended to the consumer according to his nutritional deficit. Based on the generalized model, the following potential powder mixes were highlighted and characterized by mathematical modeling in order to make food supplements: mix of garlic and wild garlic, mix of onion and wild garlic, mix of wild garlic and leek. In order to determine the moisture content, the total antioxidant capacity and the total polyphenol content of the leaves of wild garlic, onion bulbs and garlic, the following methods were used: thermogravimetric analysis, analysis of the total antioxidant capacity using the CUPRAC method, determination of the polyphenol content using the method Folin-Ciocalteu.

Key words*: Allium plants, food supplements, nutritional deficiency, powder mixes.*

INTRODUCTION

The genus *Allium* is highly diverse, encompassing approximately 1,100 taxa (Pandey, A. et al., 2022), of which 1,077 species names are currently accepted (POWO, 2025). These species are distributed almost exclusively in the Northern Hemisphere, including the Mediterranean region of Europe, Asia, North America, South America, and Sub-Saharan Africa (Vuković, S., Popović-Djordjević, J. B. et al., 2023).

The genus *Allium* holds significant economic and nutritional importance, as many of its species are consumed as vegetables, spices, or medicinal plants. Among these, *Allium cepa* and *Allium sativum* are the most widely used species, primarily serving as fundamental ingredients in various culinary preparations, as well as for medicinal purposes (Najeebullah, S. et al., 2021). Other nutritionally and economically important species include *Allium ampeloprasum* var. *porrum* (leek), *Allium fistulosum* (spring onion), *Allium tuberosum* (chinese chives), *Allium schoenoprasum* (chives), and *Allium ascalonicum* (shallots) (Vuković, S., Popović-Djordjević, J. B. et al., 2023; Poojary, M. M. et al., 2017). Most wild species are harvested by local populations and used as spices, vegetables, medicinal plants, and for ornamental purposes. These wild species constitute a valuable component of the daily human diet (Yousaf, Z. et al., 2004). Among the wild species, *Allium ursinum* is particularly noteworthy as a culinary and medicinal plant, having been used for centuries in traditional medicine. Known for its garlic-like odor, which gives it the common name "wild garlic," *A. ursinum* is appreciated for its milder flavor compared to common garlic (Piątkowska, E. et al., 2015). All parts of the plant are usable (Ivanova, A. et al., 2009), but its culinary applications are primarily limited to its leaves. The leaves are harvested early in spring, during March and April, before the plant blooms.

The edible portions of *Allium* species are versatile and can be consumed in a variety of ways, such as raw, cooked, frozen, pickled, canned, dehydrated, or incorporated into processed products (Vuković, S.; Popović-Djordjević, J. B. et al., 2023).

Most species produce significant amounts of cysteine sulfoxides, which are responsible for their characteristic aroma and flavor (Friesen, N. et al., 2006). These traits contribute to their widespread use across different fields. In the culinary domain, *Allium* species are highly valued as ingredients, commonly used as either vegetables or spices. Additionally, they are integrated into classic medicine as supplement components and have been traditionally utilized in folk medicine for the prevention and treatment of various ailments (Vuković, S.; Popović-Djordjević, J.B. et al., 2023).

The chemical composition of plants in the *Allium* genus is highly complex. The most significant constituents are organosulfur compounds (OSCs), which are primarily responsible for the distinctive odor and flavor of these plants, as well as the majority of their biological activities (Fredotović, Ž., Puizina, J. 2019; Putnik, P. et al., 2019; Poojary, M. M. et al., 2017). Another notable group of bioactive compounds includes polyphenols, such as phenolic acids and flavonoids, which contribute to the characteristic pigmentation of onion bulbs (Fredotović, Ž., Puizina, J. 2019; Carotenuto, A. et al., 1996). Polyphenols are secondary metabolites in plants that play an essential role in defending against adverse environmental conditions. Their synthesis can be intensified under various stress factors (Czech, A. et al., 2022).

In addition, plants of the *Allium* genus are rich in phytosterols and steroidal saponins (Teotia, Deepti et al., 2024; Nishimura, T. et al., 2016), fatty acids and terpenoids (Vuković, S., Moravčević, D. et al., 2023; Fredotović, Ž., Puizina, J., 2019), as well as phytoestrogens, carotenoids (Oszmianski, J., 2013; Dubouzet, J.; Shinoda, K., 1999), vitamins (A, E, C, K, B1, B3, B6, B9), and essential minerals such as phosphorus, potassium, calcium, magnesium, zinc, manganese, sodium, iron, selenium, and copper (Vuković, S.; Moravčević, D. et al., 2023; Fredotović, Ž., Puizina, J., 2019; Putnik, P. et al., 2019).

The edible parts of onion plants are rich in carbohydrates, such as fructose and glucose, while the outer layers of onion bulbs contain arabinose and galactose (Fredotović, Ž., Puizina, J., 2019). The same authors highlight the presence of essential amino acids, including glutamic acid and arginine, which serve as valuable nitrogen reserves and enhance the nutritional profile of these species.

The use of *Allium* species in both traditional and official medicine is closely associated with their rich nutritional profiles and high concentrations of phytochemicals with notable therapeutic potential. Numerous studies highlight the pharmacological properties of these species, which are directly linked to their phytochemical composition.

Wild Garlic (*Allium ursinum*)

Traditional folk medicine recommends the use of wild garlic as a remedy for fever and gastrointestinal issues (Bârlă, G. F. et al., 2016; Pavlović, D. R., 2017).

Currently, *Allium ursinum* L. and its preparations play a significant role in the prevention and treatment of cardiovascular diseases. Scientific literature indicates that the consumption of wild garlic significantly reduces blood pressure, serum cholesterol, and triglycerides (Krivokapic, M et al., 2021). Furthermore, this wild plant inhibits platelet aggregation, owing to the presence of flavonoids, and prevents the progression of atherosclerosis (Hiyasat B. et al., 2009). Additionally, it has been reported that wild garlic demonstrates a greater blood pressure-lowering effect in rats compared to common garlic (Preuss H. G., 2001).

Numerous studies have also highlighted its antioxidant (Wu, H. et al., 2009), cytostatic, and antimicrobial properties (Ivanova, A., 2009), as well as its antifungal effects (Pârvu, M. et al., 2011). The potential health benefits of wild garlic are primarily attributed to its sulfur-containing compounds. High levels of volatile compounds such as sulfides and disulfides, identified in wild garlic, directly influence its quality as both a medicinal plant and a culinary spice (Preuss H. G., 2001).

Research conducted by Xu, X. Y. (2013) confirmed that the aqueous extract of *Allium ursinum* can inhibit proliferation and induce apoptosis in AGS gastric cancer cells, suggesting that diallyl sulfides are primarily responsible for this effect in most investigated cases (Xu X.Y., 2013). Due to its content of allicin, alliin, and other sulfur compounds, the plant exhibits vermicidal, fungicidal, and

antibacterial properties (Giurgiu E., 2013; Ivanova A., 2009).
Additionally, certain studies have shown that flavonoids are responsible for inhibiting platelet aggregation in humans and possess significant antioxidant activity (Stajner D. et al., 2008). While all edible parts of wild garlic exhibit antioxidant properties, the leaves, due to their high flavonoid content, are considered the most significant in this regard (Stajner, D. et al., 2008; Oszmianski, J. et al., 2013). Consuming these vegetables, particularly in spring, can provide a beneficial supply of antioxidants.
Further research has demonstrated that *Allium ursinum* has a stronger effect in increasing high-density lipoprotein (HDL) levels, reducing total cholesterol, and lowering systemic blood pressure (Preuss H. G., 2001).
Thus, *Allium ursinum* extract holds significant potential for use in both the food and pharmaceutical industries.
Garlic (*Allium sativum*)
Numerous studies have demonstrated that garlic consumption significantly reduces total cholesterol (TC) and low-density lipoproteins (LDL), with more pronounced effects observed at lower doses over extended periods, particularly in individuals with cardiovascular diseases (Li S. et al., 2023). Raw garlic, as well as garlic extracts in the form of oil or powder, can be utilized as both functional and therapeutic foods. Substantial evidence supports the preventive and therapeutic roles of garlic in strengthening the immune system (Vuković S., Popović-Djordjević et al., 2023), exhibiting antitumor properties (Sasaki J.I. et al., 2007), and providing antioxidant activity that protects the body from free radicals (Cheng H. et al., 2020).
A balanced diet rich in functional foods incorporating garlic has been linked to numerous health benefits. Garlic has been shown to enhance the function of various organs, particularly those in the respiratory and digestive systems (Lee G.Y. et al., 2015). Both preclinical and clinical studies indicate that garlic consumption contributes significantly to antihypertensive (Hussein H.J. et al., 2017), antidiabetic (Mutlu-Ingok A. et al., 2022), immunomodulatory (Kumari N. et al., 2022), and hypolipidemic effects (Perez-Rubio K.G. et al., 2022), offering substantial benefits for both medical and surgical treatments (Daharia A. et al., 2022).
Currently, garlic is widely used worldwide as a prophylactic agent and for the treatment of various diseases, including acute and chronic infections such as pneumonia, dysentery, typhoid fever, cholera, gastritis, tuberculosis, diabetes mellitus, heart disease, and hypertension (Sashank Srivastava, S. S., & Pathak, P. H., 2012).
Onion *(Allium cepa)*
Allium cepa is commonly used to treat hypertension, bronchitis, migraines, coronary diseases, hypercholesterolemia, cataracts, and diabetes (Vuković, S., Popović-Djordjević et al., 2023).
Leek (*Allium ampeloprasum*)
Although its therapeutic effects are less pronounced compared to other *Allium* species, leek (*Allium ampeloprasum*) has been used since ancient times to treat a wide range of ailments. The scientific literature highlights notable health benefits of *Allium ampeloprasum*, including antibacterial, antifungal, antioxidant (Garcia-Herrera P. et al., 2014), vermifuge and antihypertensive (Guarrera P. M., Savo V., 2013).
Other studies have revealed its antitoxic, anti-inflammatory, and immunostimulatory properties (Dey P., & Khaled K.L., 2015). Leek also helps reduce the risk of gastrointestinal diseases and protects the skin from damage (Strati I.F. et al., 2018).
The aim of the study was to characterize the main bioactive compounds in onion, leek, garlic and wild garlic plants in order to obtain new data and information that would lead to highlighting the phytopharmaceutical potential of these plants. Another objective was to characterize potential mixes of dried plants through mathematical modeling in order to create food supplements.

MATERIALS AND METHODS

Allium cepa, Allium ampeloprasum and *Allium sativum* used in the experiments were sourced from gardens located in an unpolluted region of Timiș County, while *Allium ursinum* was harvested from a hilly area.
All reagents were of analytical grade.
All analysis were performed in triplicate.

The performed analyses were: thermogravimetric analysis, total antioxidant capacity based on CUPRAC method, and total polyphenol content based on Folin-Ciocalteu method.

Thermogravimetric analysis

The moisture content was determined using the Sartorius thermobalance monitoring the dehydration process of the studied plant material through continuous weighing.

CUPRAC Antioxidant Reduction Method

The method is based on the protocol described by Apak et al., 2004. The method is applicable to both hydrophilic and lipophilic antioxidants (unlike Folin and DPPH tests) and presents selective action towards antioxidant compounds without affecting sugars and citric acid, which are commonly present in food products. The method has the advantage of linearity across a broader concentration range and can also determine the antioxidant activity of substances containing sulfhydryl (-SH) groups, such as proteins (unlike FRAP) (Apak R., 2007). The CUPRAC reagent does not involve any radical reagent, making it insensitive to several physical parameters such as temperature, sunlight, pH, humidity, etc., and it can be adsorbed onto a cation-exchange membrane to construct a sensor with a linear antioxidant response (Bener M. et al., 2010).

***Determination of Total Phenolic Content* (TPC)**

The method is a colorimetric method described by Folin-Ciocalteu. with slight modifications. As standard was used Gallic acid, and the results were expressed as mg gallic acid equivalents (GAE) per 100 mL of extract (Nuncio-Jáuregui N., 2015).

The experimental determinations were performed on 50% alcohol extracts obtained from wild garlic leaves, garlic bulbs, and onion bulbs, slowly dehydrated at temperatures between 40-60°C.

Creation of a compositional database based on literature data, in order to identify the main patterns of garlic, wild garlic, onion and leek, nutritional and phytopharmaceutical compounds.

Statistical processing

The interpretation of the obtained data was carried out using: simple and logarithmic Barchart diagrams, cluster analysis, linear correlation r and mathematical modeling. The generalized linear model was carried out using the PAST and MVSP software.

RESULTS AND DISCUSSIONS

The results are presented in Table 1 and Figure 1.

Table 1. Samples analysis results

Dehydrated samples	Moisture [%]	TAC [mg TE.100 g^{-1} d.w.]	TPC [mg TE.100 g^{-1} d.w]
Wild garlic	89.02	433.2	361.2
Onion	65.52	523.4	302.7
Garlic	27.33	632.6	265.5
Leek	63.85	588.8	295.6

From Table 1, it is observed that the highest moisture content (U%) and total polyphenol content (TPC) are found in wild garlic leaves, while garlic exhibits the highest total antioxidant capacity (TAC), data that are also confirmed by the scientific literature (Bârlă G.F., 2016; Ivanova A., 2009; Piątkowska E., 2015; Sobolewska D., 2015).

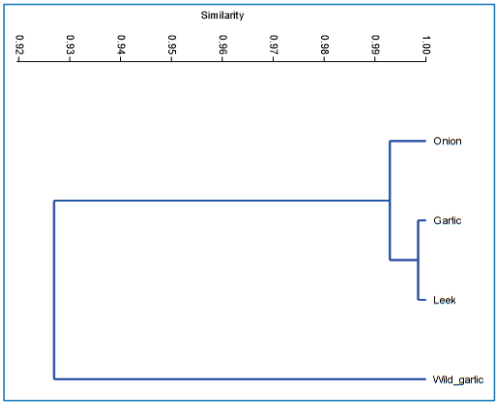


Figure 1. Cluster analysis of data

The cluster analysis applied to the experimental data highlights a correlation coefficient of 0.8052 (Figure 1).

The cluster analysis based on the associated grouping algorithm and similarity correlation highlights a high similarity of the analyzed extracts. It is observed that the extract from garlic and leek shows a greater similarity than those of onion and wild garlic.

This high correlation demonstrates a strong similarity among the samples concerning their nutritional and phytopharmaceutical properties.

Fingerprinting (Figure 2) using the MVSP software highlights the particularities of the wild garlic alcoholic extract, which can stabilize alcoholic extracts based on onion or garlic.

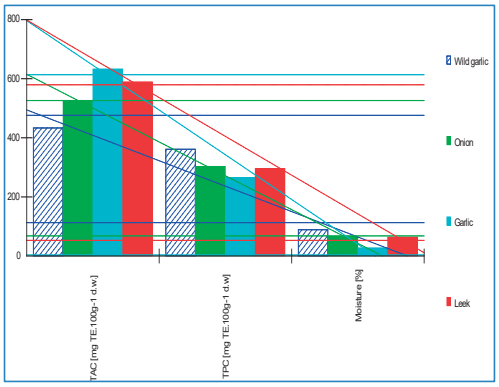


Figure 2. Fingerprint of wild garlic, onion, and garlic alcoholic extracts

The laboratory results were completed with the study of literature data to create a complex database of chemical and nutritional parameters of the analyzed plants (garlic, wild garlic, onion and leek) and the results were graphically represented in Figures 3 and 4. The interpretation of the results were carried out using PAST software.

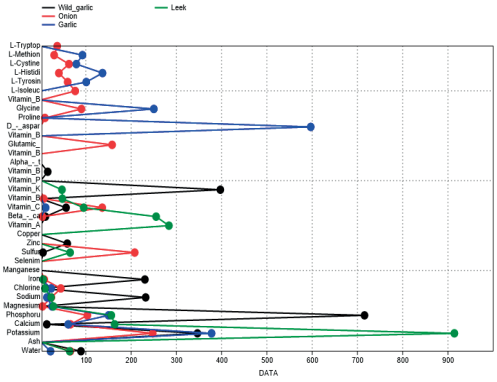


Figure 3. Graphical representation of the investigated data values

Figure 3 shows that wild garlic (*Allium ursinum*) has the highest content of water, vitamin K1, B2, iron, sodium, phosphorus, and zinc. Onion (*Allium cepa*) has the highest content of chlorine, sulfur, vitamin C, glutamic acid, L-isoleucine, and L-tryptophan. Garlic (*Allium sativum*) is characterized by its high selenium content, vitamin B6, D-aspartic acid, glycine, and the highest levels of amino acids (L-Tyrosine, L-Histidine, L-Cysteine, L-Methionine). Leek (*Allium ampeloprasum*) is recommended for its highest content of potassium, calcium, magnesium, vitamin A, and vitamin C.

The Barchart diagram (Figure 4) allows us to identify the nutritional components representative for each product investigated (black for wild garlic, red for onion, blue for garlic, and green for leek).

This method enables us to create dehydrated powder mixtures based on the nutritional deficiencies of the consumer. Specifically, individuals with an amino acid deficiency can consume a product with a high content of garlic powder, while those with a deficiency in minerals and vitamins are recommended a mixture of leek and wild garlic powders.

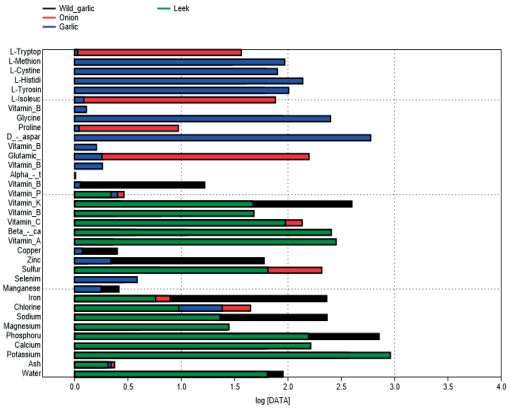


Figure 4. Barchart diagram of the phytopharmaceutical characteristics

To further understand the positioning of wild garlic (*Allium ursinum*) in comparison to garlic (*Allium sativum*), onion (*Allium cepa*), and leek (*Allium ampeloprasum*) in terms of their physicochemical characteristics, a correlation analysis (r) was performed (Table 2). The purpose of the correlation analysis is to provide insights into the strength and direction of the linear relationship between two variables, which is crucial for identifying the most suitable formulations based on specific nutrient profiles (https://www.dummies.com/education/math/statistics/how-to-interpret-a-correlation-coefficient-r/)

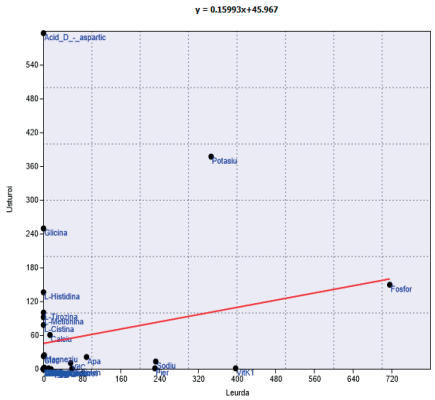
Table 2. Linear correlation r

Product	Wild Garlic	Onion	Garlic	Leek
Wild Garlic	0	0.1023	0.2623	0.0482
Onion	0.2807	0	0.0921	0.0045
Garlic	0.1947	0.2892	0	0.0627
Leek	0.3363	0.4686	0.3179	0

From the perspective of phytopharmaceutical and nutritional characteristics, bear's garlic leaves show a positive correlation with garlic (0.2623), indicating an ascending relationship between the two.

y = 0.159993x+45.967

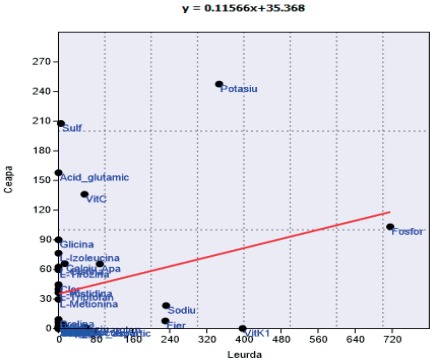
x = amount of wild garlic [mg]
y = quantity of garlic [mg]



5.1. Garlic and wild garlic mix
The product is characterized by a high content of Phosphorus, Potassium, and Aspartic Acid.

y= 0.11566x+35.368

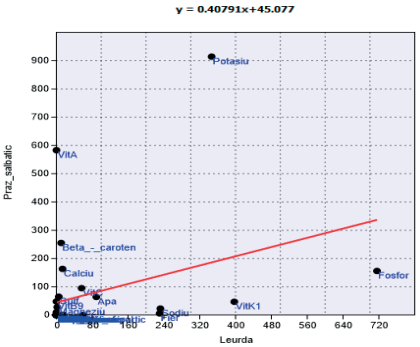
x= amount of wild garlic [mg]
y= amount of onion [mg]



5.2. Onion and wild garlic mix
The mix of wild garlic and onion is characterized by a high content of Phosphorus, Potassium, and Sulphur.

y= 0.40791x+45.077

x= amount of wild garlic [mg]
y=quantity de wild leek[mg]



5.3. Wild leek and wild garlic mix
The mix is characterized by a high content of Phosphorus, Potassium, and Vitamin A,

Figure 5. Characterization of potential dried plant mixtures through mathematical modeling

The generalized linear model was created using the PAST program based on the normal distribution and the identity function, with the estimation of the phi coefficient.
The application of generalized linear models highlights the following potential powder mixtures for the formulation of dietary supplements (Figure 5).

CONCLUSIONS

The analysis of the nutritional composition of the investigated species yielded several significant observations:

- *Allium ursinum* (wild garlic) exhibited the highest levels of water, vitamin K_1, vitamin B_2, iron, sodium, phosphorus, and zinc among the studied samples.
- *Allium cepa* (onion) was notable for its elevated content of chlorine, sulfur, vitamin C, glutamic acid, L-isoleucine, and L-tryptophan.
- *Allium sativum* (garlic) was distinguished by its particularly high concentrations of selenium and vitamin B_6, as well as D-aspartic acid and glycine. Furthermore, it contained the greatest overall abundance of amino acids, including L-tyrosine, L-histidine, L-cysteine, and L-methionine.
- *Allium ampeloprasum* (leek) demonstrated the highest levels of potassium, calcium, and

magnesium, and was also a valuable source of vitamins A and C.

- The bar chart analysis, which facilitated the identification of the predominant nutritional constituents of each species, provides a practical framework for the development of functional dehydrated powder blends tailored to individual nutritional needs. For example, garlic-based powders may be particularly suitable for individuals with amino acid deficiencies, while mixtures incorporating leek and wild garlic powders are recommended for those with insufficient mineral and vitamin intake.

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THE EFFECT OF GROWING CONDITIONS ON THE YIELD AND QUALITY OF LETTUCE CULTIVATED IN THE NFT SYSTEM

Emanuela COJOCARU (JERCA), Sovorn CHAN, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: draghiciem@yahoo.com

Abstract

This research investigates the effects of culture conditions on the quality of 8 lettuce varieties grown in a Nutritive Film Technique (NFT) system under LED lighting. The NFT system, an advanced hydroponic method suitable for growing cycle leafy greens, offers commercial advantages while enhancing both the nutritional and biochemical value of crops. The main aim of this study was to evaluate the growth and development of lettuce varieties in the NFT system under controlled LED lighting conditions. The study was carried out within the Faculty of Horticulture, UASMV Bucharest, under LED lighting conditions, in the research space of the Plant Factory. Vertical farms, also known as plant factories, optimize agricultural production per unit area by arranging crops on multiple stacked levels. This method is particularly advantageous in urban environments, where space availability is limited. These facilities operate year-round, being weather-independent and ensuring constant production. Although they save space and water, they consume a lot of energy for lighting and climate control, increasing costs and environmental impact.

Key words*: lettuce, LED lighting, fertilization, biochemical content.*

INTRODUCTION

Urbanization contributes to social and economic transformations, and the conversion of land from agricultural to non-agricultural activities has led to the development of new cultivation technologies (Frasetya et al., 2021).

Urban agriculture employs modern technologies in the NFT system (Chan et al., 2023) as well as perlite substrate (Micu et al., 2022) to achieve rapid and healthy yields, as well as high incomes due to reduced production costs (Micu et al., 2023). This can be accomplished through both traditional and modern methods but requires advanced technologies for high yields on limited spaces. The preference for leafy vegetables, especially pesticide-free lettuce, has accelerated the development of hydroponics in cities.

To reduce soil pollution with nitrates, one essential measure is the application of fertilizers according to the actual needs of the plants and the amount of nitrogen already present in the root zone prior to fertilization (Marinov et al., 2010).

Kowalczyk et al. (2016) emphasize the growing interest in hydroponic lettuce production, highlighting the influence of different growing media on yield and biological value. They noted that the NFT system produced the highest yield, while plants grown on coconut fiber had the lowest dry matter content.

The use of organic fertilizers in the NFT system led to the production of lettuce plants with a reduced nitrate content (Drăghici et al., 2016). Additionally, the use of structured water contributed to increased yields as well as beneficial microbiological activity (Enache et al., 2019; Matei et al., 2023).

Electrical conductivity affects the growth and quality of lettuce plants in NFT culture (Abou-Hadid et al., 1996; Nguyen et al., 2018), and Sanguandeekul (1999) notes that low electrical conductivity, particularly at night, leads to lower nitrate accumulation.

Based on conducted studies, Singe et al. (2015) concluded that cultivating lettuce in the NFT system does not affect its content (Singer 2015; William et al., 2018; Drăghici et al., 2016).

Urechescu et al. (2022) mention that the use of vermicompost for growing lettuce seedlings intended for unconventional systems led to an improvement in their quality.

Sadhana et al. (2022), in their study, concluded that the use of perlite resulted in plants with

higher average masses compared to Cocopeat, Sponges, or other substrates in NFT cultivation. Panter et al. (2014) highlighted the quantitative and qualitative differences in seedlings and lettuce production under LED lighting.
In the NFT system, a nutrient solution circulating at a specific flow rate and with a higher level of oxygenation leads to better growth and development of the root system, with beneficial effects on lettuce yield and quality (Stoica Claudia Maria et al., 2022).
The aim of the study was to highlight the effect of the NFT cultivation technology using natural light and LED lighting on both the yield and quality of the produce.

MATERIALS AND METHODS

The experiment was conducted in Faculty of Horticulture, USAMV, Bucharest, and in Plant Factory greenhouse, this being built within a joint project to create a China-Romania Demonstration Park for Science and Technology in Agriculture, partly financed by the Chinese Academy of Agricultural Sciences and partly by USAMV from Bucharest.
Eight varieties of lettuce collected in Romania were used in the research only under LED red light and in greenhouse under natural light: V1 - 'Lollo Bionda'; V2 - 'Carmesi'; V3 - 'Aleppo'; V4 - 'Lugano'; V5 - 'Presteria'; V6 - 'Tourbillom'; V7 - 'Triathlom'; and V8 - 'Sandalina'.
The temperature in the Plant Factory culture space was 20 ± 2ºC, and the nutrient solution was 19-20ºC. The duration of illumination was 18 hr/6 hr of light/dark with constant intensity of 181.7 µmol/m^2s^1. In the greenhouse, under natural conditions, the light intensity was variable, around 274.3 µmol/m^2s^1. The EC was 1.2 mS/cm in the first growth phases and of 2.2 mS/cm after two weeks from planting and the pH was kept constant at 6.
All data were recorded during the harvest period, in repetitions of 5 plants each. The following were determined: plant height, diameter, plant mass, root length and root volume. In laboratory, for the dry matter, 1 gram of fresh leaves was used by cutting the leaves into small pieces and then dried at a constant temperature of 105ºC for 24 hours.
Brix content using reflectometer, and nitrate using portable nitrate Greentest ECO. The values obtained for the basal, middle, and upper leaves of lettuce plants, for each cultivar, were recorded.
The statistical program for analysis was STATISTICA, StatSoft software (version 10) to perform ANOVA analysis at $p \leq 0.05$, 0.01, or 0.001 levels, and Tukey HSD was used to compare the significant difference of each dependent variable at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

In the greenhouse, the minimum temperatures during the night showed minor fluctuations, ranging from 16.8°C at 15 days to 17.6°C at 20 days. The overall average of these minimum temperatures throughout the growing period was 17.1°C. During the day, a slight increase in the average temperature was observed in the first four intervals, reaching 21.6°C at 20 days, followed by a decrease to 19.4°C at 25 days, and then returning to 20.7°C at 30 days. The average for these intervals was 20.5°C, indicating a moderate temperature. This value suggests that the conditions in the greenhouse were relatively stable, although there were some variabilities between different time intervals.
The maximum temperature values showed a more pronounced variation compared to the minimum and average temperatures, peaking at 25.6°C at 20 days, then significantly dropping to 21.8°C at 25 days, before rising again to 24.3°C at 30 days. The overall average of the maximum temperatures was 23.8°C (Figure 1).

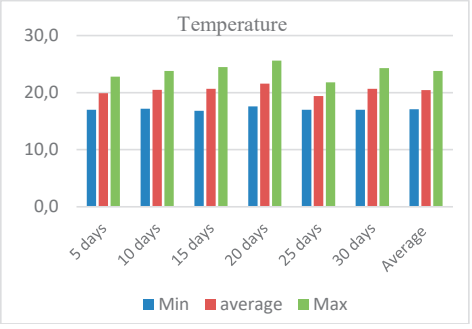


Figure 1. The temperature values recorded in the greenhouse

The minimum humidity recorded during the night was relatively stable, fluctuating between 33.0% and 36.5%. The lowest minimum value, 33.0%, was observed after 5 days, while the highest minimum value, 36.5%, was reported at 30 days. The average humidity showed an upward trend throughout the growth of the plants in the greenhouse. The atmospheric humidity was 39.5% after 5 days, reaching 47.4% at 30 days, with a value of 49.1% recorded at 25 days. Additionally, the maximum humidity values reached 62.2% at 25 days (Figure 2).

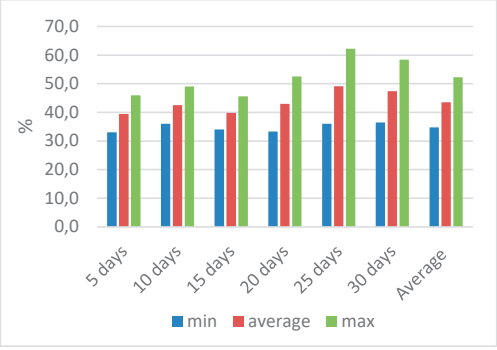


Figure 2. The values of the atmospheric humidity recorded in the greenhouse

The minimum, average, and maximum CO_2 values fluctuated significantly over the 30 days. A general upward trend was observed in the average values, rising from 529.7 ppm at the measurement taken after 5 days to 625.1 ppm after 30 days. The highest value recorded was 918.4 ppm at the measurement taken after 25 days. The minimum values varied between 387.7 ppm and 427.3 ppm (Figure 3).

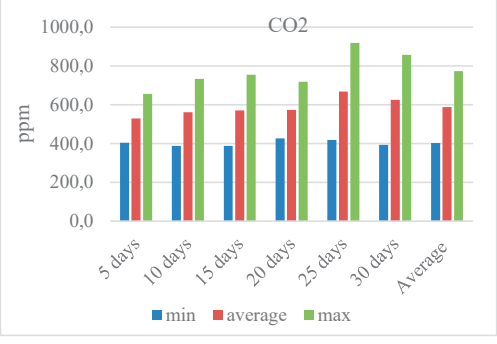


Figure 3. The carbon dioxide values recorded in the greenhouse

For the Plant Factory, the nutrient solution was kept at a constant temperature of 20ºC and exposed to light continuously for 16 hours, followed by an 8-hour break with constant intensity of 179.7 µmol/m^2s^1. Throughout the entire cycle, the concentration of carbon dioxide (CO2) was maintained at 600 (ppm).
The pH of 6 and electrical conductivity (EC) from 1.2 to 2.2 micro-Siemens/cm of the nutrient solution were continuously monitored and maintained at optimal values
On average, plants grown in the 'Plant Factory' were taller than those grown in the 'Greenhouse'. This suggests a superior efficacy of the controlled conditions in the 'Plant Factory' for growing lettuce plants. The tallest height recorded in the 'Plant Factory' was for 'Alepo', measuring 24.50 cm, while in the 'Greenhouse' the tallest height is also for 'Aleppo', with 21.45 cm. The shortest height recorded in the 'Plant Factory' is for the 'Lugano' plant, with 18.17 cm, and in the 'Greenhouse', the shortest height is for 'Presteria', with 16.33 cm. The average growth difference between the two cultivation mediums varies significantly from one variety to another, indicating that certain varieties may be more sensitive to environmental conditions than others. The variability in plant height between the two mediums is notable, suggesting that the environmental factors specific to each type of growth medium have a significant impact on plant growth (Figure 4).

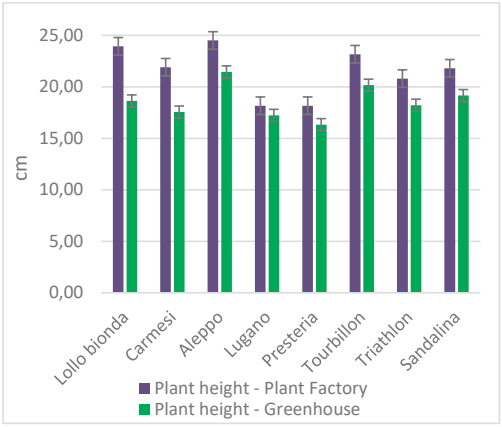


Figure 4. The height of the lettuce plants in the greenhouse and plant factory

These observations indicate that the different growth conditions in the two environments

have a variable impact on the diameter of the plants. Some varieties seem to benefit from the controlled environment of a plant factory, while others grow better in the potentially more varied conditions of a greenhouse. The 'Lollo Bionda' variety has a considerably larger diameter in the greenhouse (37.17 cm) compared to the plant factory (28.17 cm), suggesting that this variety may be more responsive to the conditions in the greenhouse to expand its diameter. The 'Carmesi' variety shows a better diameter growth in the greenhouse (32.83 cm) than in the plant factory (30.22 cm), but the difference is not as pronounced as for ''Lollo Bionda''. 'Aleppo' shows large diameters in both environments, being very close (35.55 cm in the plant factory and 37.00 cm in the greenhouse), suggesting that this variety grows well under both cultivation conditions. The 'Lugano' variety has an almost identical diameter in both locations, with a minor difference of only 0.20 cm in greenhouse conditions. For 'Presteria', there is a larger diameter difference, with greenhouse plants having almost 2.5 cm more than those from the plant factory. 'Tourbillom' has a significantly larger diameter in the greenhouse (34.00 cm) compared to the plant factory (30.22 cm), similar to observations for 'Lollo Bionda'. 'Triathlom' is one of the few varieties that has a larger diameter in the plant factory (31.17 cm) than in the greenhouse (30.55 cm). The variety 'Sandalina' has a slightly larger diameter in the plant factory (34.17 cm) compared to the greenhouse (33.18 cm) (Figure 5).

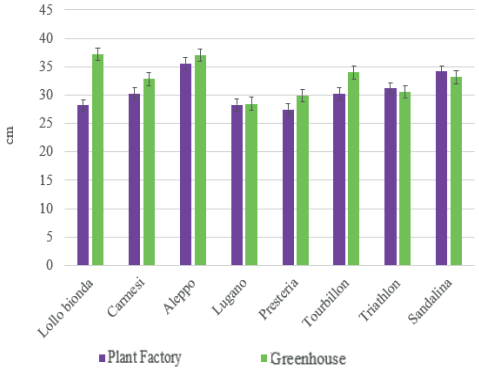


Figure 5. Diameter of lettuce plants grown in the greenhouse and plant factory

Each variety is evaluated based on two parameters: Fresh weight and percentage of dry matter. The table provides specific data on fresh weight and the percentage of dry matter for eight plant varieties grown in a plant factory and a greenhouse. For example, the variety 'Lollo Bionda' has a fresh weight of 139.87 grams in the Plant Factory, compared to 118.32 grams in the greenhouse, and a dry matter percentage of 5.65% in the plant factory versus 5.21% in the greenhouse. This indicates that 'Lollo Bionda' grew better in the controlled conditions of the plant factory. Comparatively, the 'Sandalina' variety has the highest fresh weight in the Plant factory (227.66 g), but in the greenhouse, its fresh weight significantly decreases (182.05 g), indicating that this variety might particularly benefit from the conditions in the plant factory. However, for the dry matter percentage, 'Sandalina' has a lower percentage in the plant factory (4.84%) compared to the greenhouse (5.03%), which could suggest a different composition of the plant's matter in the two environments (Figure 6).

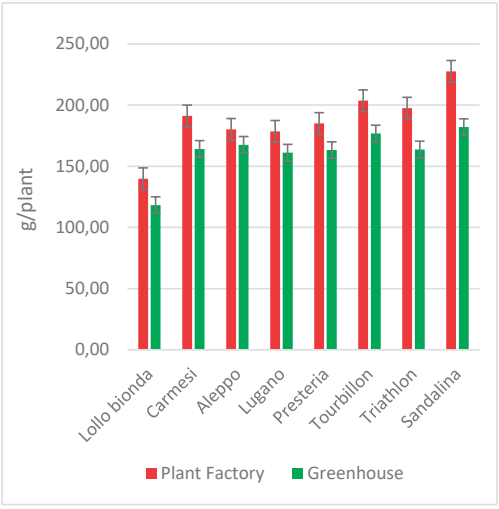


Figure 6. Average mass of lettuce plants grown in the greenhouse and Plant factory

The root length was influenced by environmental factors. With a constant temperature of the nutrient solution at 20°C, an improved root development was observed, which positively impacted the growth of the plant's mass (Figure 7).

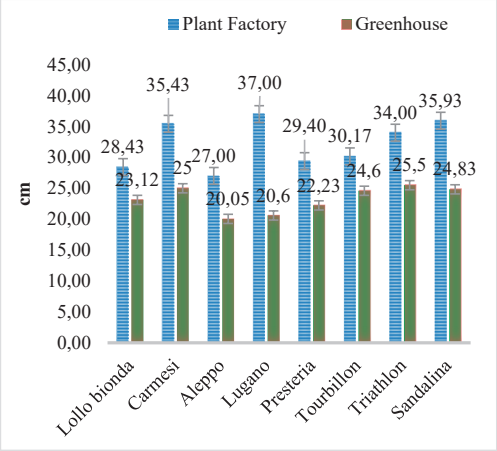


Figure 7. Results regarding the length of the roots

Based on the data presented in the figure 8, we can observe that depending on the cultivation location (Plant Factory and greenhouse), the dry matter content varied for some varieties. Thus, for the varieties 'Lollo Bionda', 'Carmesi', 'Aleppo', 'Lugano' 'Presteria', and 'Tourbillon', the determined dry matter content was higher under Plant Factory conditions compared to the greenhouse. In contrast, for the varieties Triathlon and 'Sandalina', the dry matter content was lower compared to the plants grown in the greenhouse (figure 8).

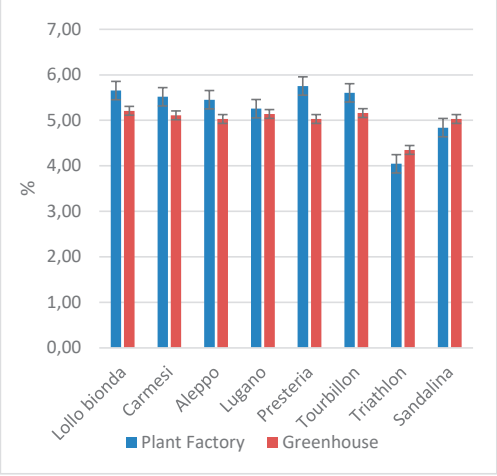


Figure 8. Dry matter content of lettuce plants

Figure 9 shows the appearance of plants in culture, in the greenhouse and in the Plant Factory.



Figure 9. Appearance of plants in culture

The nitrate content was below the maximum limit allowed by the legislation of 3000 mg/kg in the case of all the analyzed varieties. Large differences were found in the nitrate content of the leaves, thus, the basal leaves showed high values of the nitrate content compared to the middle leaves and those located at the top of the lettuce plant (Figures 10 and 11).

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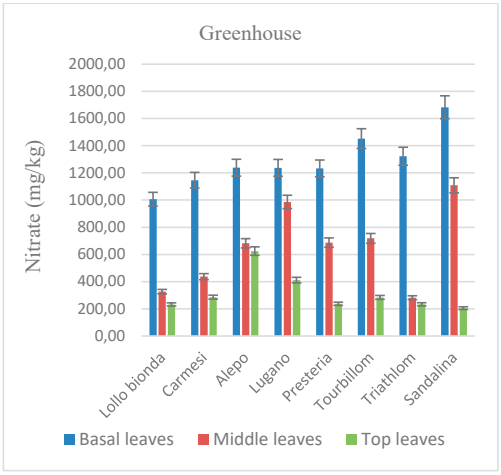


Figure 10. Nitrate content determined in greenhouse-grown lettuce plants

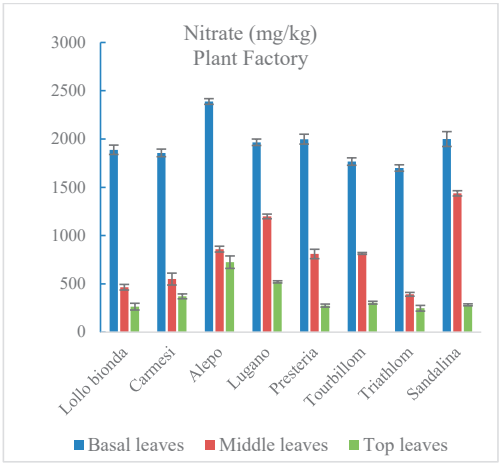


Figure 11. Nitrate content determined in Plant Factory grown lettuce plants

Regarding the carbohydrate content, in general, Brix content was high in the top and middle of the leaves, while nitrate was abundant in the basal leaves of the lettuce (Figure 4). It has been observed that the base leaves have the highest carbohydrate levels, with the 'Sandalina' variety having 6.9 g and 'Presteria' 6.4 g, which are the maximum values. The intermediate leaves exhibited greater variability, with the 'Carmesi' variety having the highest concentration of 4.3 g. Regarding the upper leaves, they have a low carbohydrate content, most of them being below 3.0 g, with the exception of the 'Sandalina' variety, which has 7.8 g (Figure 12).

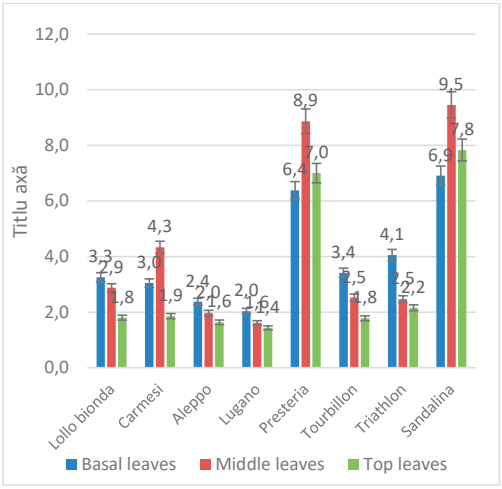


Figure 12. Carbohydrate content in greenhouse-grown lettuce plants

Compared to the carbohydrate values recorded in greenhouse plants, it was observed that under LED lighting conditions, the basal leaves had a lower carbohydrate content, while the middle and top leaves exhibited higher values, Figure 13.

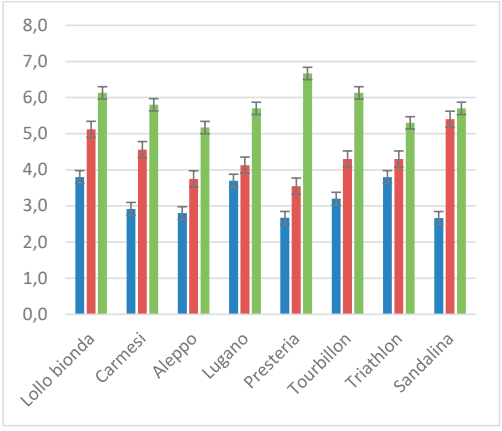


Figure 13. Carbohydrate content in Plant Factory grown lettuce plants

CONCLUSIONS

The evaluation of fresh weight and dry matter percentage reveals that the 'Lollo Bionda' variety thrives in the controlled conditions of the plant factory, while 'Sandalina' shows higher fresh weight in the plant factory, but lower dry matter percentage compared to the greenhouse. This suggests that different

environments affect not only the growth performance but also the composition of the plant matter for various lettuce varieties.

Lettuce plant diameter varies significantly between cultivation environments. The ''Lollo Bionda'' and 'Tourbillon' varieties thrive in greenhouses, while 'Triathlon' grows better in the plant factory. 'Carmesi' and 'Presteria' also show improved growth in greenhouses, whereas 'Aleppo' performs well in both settings. These results emphasize the importance of selecting appropriate environments for different lettuce varieties to maximize growth.

The cultivation location significantly influences the dry matter content of lettuce varieties, with most varieties ('Lollo Bionda', 'Carmesi', 'Aleppo', 'Lugano' 'Presteria', and 'Tourbillon') achieving higher dry matter levels in the Plant Factory compared to the greenhouse. However, the Triathlon and 'Sandalina' varieties displayed lower dry matter content in the Plant Factory, highlighting the importance of environmental conditions on plant development.

The nitrate content in all analyzed varieties was below the legislative maximum limit of 3000 mg/kg. Notable differences were observed in nitrate levels among the leaves, with basal leaves exhibiting significantly higher nitrate concentrations compared to the middle and upper leaves of the lettuce plant.

The carbohydrate content in lettuce varies by leaf position, with the highest levels found in basal leaves of the 'Sandalina' and 'Presteria' varieties, while upper leaves generally have lower carbohydrate levels, except for the 'Sandalina' variety.

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ENHANCING FRUIT YIELD AND QUALITY IN PEPPER AND EGGPLANT: THE ROLE OF MULCHING TECHNIQUES

Delia-Cristina CONSTANTIN, Mihaela PARASCHIV

Research and Development Institute for Vegetable and Flower Growing Vidra,
22 Calea București, Vidra, Ilfov, Romania

Corresponding author email: mihaela1mail@yahoo.ca

Abstract

Soil mulching can create a microclimate at the plant level and has several advantages, the most important being its inhibitory effect on weed growth and its ability to retain soil moisture for a longer period. The purpose of this study is to determine the influence of different types of agricultural mulching films on yield and fruit quality of round pepper and eggplant. The experiment took place at RDIVFG Vidra in 2024. The biological material for peppers consisted of seedlings of the round pepper cultivar Asteroid 204 and eggplant cultivar Belona. Four variants were studied: unmulched soil, mulching with black foil, mulching with white-black foil, and mulching with brown foil. It was found that the use of mulch has beneficial effects on the yield and quality of fruits both in pepper and eggplant. Among the mulching options, the white-black foil resulted in the greatest increases in the number of fruits per plant, yield, and fruit weight in peppers, also positively influencing fruit quality. In eggplant, the greatest positive effects were observed with the use of brown foil.

Key words*: Capsicum annuum, fruit quality, mulch, Solanum melongena, yield.*

INTRODUCTION

In Romania, it has been observed that drought is not a constant and uniform phenomenon throughout the country. Rather, some areas are more exposed to the risk of drought during the hottest summer months. The areas most affected by drought stress are the southern and northeastern regions of the country, with the west also exposed, although with lower probability (Dobri et al., 2021).

Soil mulching is a maintenance practice often employed in vegetable crop, both in protected environments and in open fields. This practice involves covering the soil surface between plants with organic or synthetic materials (Choudhary et al., 2022; Ciofu et al., 2004; Dinu, 2022), leading to a specific microclimate around the plants that differs from that characteristic of unmulched soil (Tarara, 2000).

The main effects of mulching are reflected in the properties of the soil: it retains soil moisture for a longer period, serves as an effective method for controlling weeds, and helps maintain soil temperature (Choudhary et al., 2022; Ranjan et al., 2017). Due to its direct effect on soil moisture conservation by preventing water evaporation, mulching is widely used in arid regions (Kazemi and Safari, 2018). Additionally, some studies have indicated that the practice of covering land surfaces with mulch and utilizing drip irrigation can enhance the efficiency of nitrogen use by plants (Vázquez et al., 2006).

Soil mulching significantly influences the incidence and transmission of soil-borne diseases (Choudhary et al., 2022), suppressing the development of some diseases while favoring the spread of others (Ngosong et al., 2019).

Moreover, mulch affects the spectrum of reflected light (Kaul and Kasperbauer, 1992). Another important aspect is the potential of mulch in the biocontrol of certain vegetable pests (Choudhary et al., 2022; Ngosong et al., 2019).

Mulch positively influences earlier fruiting of vegetables and can improve fruit quality and yield (Choudhary et al., 2022).

Among synthetic materials, plastic films are the most widely used, which can come in various colors: black, white, gray (Dinu, 2022), brown, green (Johnson and Fennimore, 2005), yellow, red, blue (Manzoor et al., 2023) or combinations of two colors, such as black on one side (toward the ground) and red or another

silver color on the other side (Barche et al., 2015). Different colored mulches have specific effects on the microclimate created and, therefore, different influences on fruit production and quality (Choudhary et al., 2022; Manzoor et al., 2023; Mutetwa and Mtaita, 2014; Tarara, 2000).

One of the most commonly used types of plastic mulch is black (Amare and Desta, 2021; Lalitha et al, 2010), which increases soil temperature by transferring energy absorbed from the environment (Strik et al., 2017; Tarara, 2000). This increase in temperature affects not only the physical and chemical properties of the soil but also its pH level, thereby influencing the availability of essential nutrients for plant growth (Strik et al., 2019).

White mulch can produce the opposite effect of black mulch (Amare and Desta, 2021; Tarara, 2000). In the case of black-and-white mulch, the upper (white) layer is designed to reflect sunlight, helping to prevent soil overheating during periods of high temperatures, while the lower (black) layer effectively controls weeds due to its ability to absorb heat (Granatstein & Sanchez, 2009). Furthermore, transparent mulch, despite its limited ability to absorb solar radiation, is less effective for weed suppression (Zhang et al., 2021).

Regarding certain soil diseases, a positive effect has been observed in terms of decreasing their incidence when using black film mulching (Abdallah et al., 2019).

The aim of this study was to investigate the influence of various types of plastic mulch films on the production and quality parameters of round pepper and eggplant fruits.

MATERIALS AND METHODS

The experiment took place in an unprotected field located within the Research and Development Institute for Vegetable and Flower Growing Vidra, Ilfov County, in the south of Romania (44°16'22.5"N 26°10'11.7"E), between May - October 2024.

The biological material consisted of seedlings of round pepper cultivar Asteroid 204 and the eggplant cultivar Belona, two varieties of which the institute is the author and maintainer. Seedlings were produced in alveolar trays with a volume of 50 ml, in fertilized peat substrate with a pH of 5.5-6.5 (Klasmann TS3 product).

Three different types of mulch films were compared with each other and with the unmulched variant. The types of plastic mulch film studied in the present experiment were the following: black, white-black and brown mulch film. The field was prepared prior to planting by applying the plastic mulch film, according to the experimental variants, which were placed in split-plot design. Each variant had four repetitions, resulting a number of 16 experimental plots, with a surface area of 13 m^2 each.

The planting was carried out on 17th of May, 2024. The seedling was 60 days old, in both species.

The seedlings were planted in two rows on each bed, with a distance of 70 cm between rows. The distance between plants was 25 cm, for round pepper seedlings, and 40 cm for eggplant. The plants were watered by drip irrigation.

During the growing season, two manual and two mechanical weedings were carried out.

Table 1 presents the climatic conditions in which the research was carried out.

According to a press release issued by the National Meteorological Administration of Romania (2025), the year 2024 was the warmest year in the history of meteorological measurements in the country, and the summer of 2024 was the warmest in the period 1900-2024.

In the conditions of such a year, it is necessary to adapt crop technologies to current climatic conditions.

Irrigation was carried out in the same way for all variants, without considering the presence or absence of mulch. Watering was carried out 3 days apart, except for rainy days, with a watering rate of 55 m^3/ha. In periods with temperatures exceeding 32OC, watering was carried out with a rate of 65-70 m^3/ha.

The round pepper fruit was harvested at technological maturity, which coincides with physiological maturity. The technological maturity of eggplant differs from physiological maturity. Due to the strong influence of the mulched variants compared to the unmulched one, harvesting was differentiated, depending on the time at which each variant reached

technological maturity. Diseased or blossom end rot affected fruits were removed. As production indices, were determined the yield, the fruit weight and number of fruits per plant. Several analyses were carried out in the laboratory regarding the quality of round pepper and eggplant fruits. It was determined total of water content in fruit, total dry matter content, total dry soluble substance content, ash content. For each experimental variant, each determination was performed three times. The water and dry matter content of the fruit pulp was expressed in percentages. The gravimetric method was used to determine the water and dry matter content. For this purpose, 10 g of sample were dried in an oven at a temperature of 105°C until constant weight was reached (Krełowska-Kułas, 1993). Total soluble solids content (TSS) was determined with an Abbé refractometer and was expressed as °Brix (PN-90/A-75101/02).

The ash content was determined by calcining the samples at a temperature of 450°C, for 48 hours and was expressed as a percentage of fresh weight (% FW) (Horwitz, 2000).

The influence of experimental factors was analyzed using IBM SPSS Statistics for Windows, Version 26. The significance of differences between variants was evaluated using the Duncan's Multiple Range Test performed at $P \leq 0.05$.

Table 1. Crop climatic conditions (Vidra, Ilfov County, Romania, 2024)

		Temperature (^{O}C)			Relative air humidity (%)			
		Medium	Max	Min	Medium	Max	Min	Rainfall (mm)
May	I	16.05	22.2	10	60.1	89	32.9	8
	II	14.9	20.6	8.7	58.1	89.6	30.2	6
	III	18.58	25.55	12	56.45	86.09	26.55	6
June	I	24.72	32.4	16.6	52.5	90	23.8	4
	II	25.34	32	18.4	52.8	84.9	26.4	12
	III	25.02	31.64	19.09	56.91	88.27	27.36	42
July	I	26.44	33.2	19.1	42.8	76.1	21	24
	II	30.43	37.9	22	37.9	74.1	15.4	11
	III	25.02	31.64	19.09	56.91	88.27	27.36	42
August	I	25.98	31.5	17.6	53.1	86.4	27.2	0
	II	28.05	35	20.6	39.3	65.3	18.9	0
	III	25.83	33	19.55	41.45	68.36	19.73	0
September	I	23.13	30.2	15.9	42.1	72	20.5	0
	II	17.8	22.8	13.4	70.5	94.2	41.7	6
	III	19.24	26.4	13.1	61.8	91.4	30.2	40
October	I	16.04	23.4	10.5	74.3	99.4	39.7	10
	II	11.97	18.1	7.1	68.9	92.6	39.4	3
	III	10.58	19.27	3.82	70.73	98.36	33.27	0

RESULTS AND DISCUSSIONS

Figure 1 shows how the experimental variants influenced the phenological development stages at round pepper plants. In round pepper, technological maturity coincides with physiological maturity. The fruits were harvested at physiological maturity (BBCH85 and BBCH88).

The developmental stages of pepper plants were strongly influenced by the mulched variants, compared to the unmulched variant.

In round peppers, the first flower opened (BBCH 61) between 5th-10th of June, in the case of mulched variants. The unmulched variant led to a delay of 13.5-17.25 days. The significant differences are due to the fact that in the case of the variant without mulch, water was lost from the soil more quickly through evaporation, and the plants developed slowly.

Also, the variant without mulch led to delayed in fruit setting and ripening. The discrepancy between the no-mulch and mulched variants became even greater over time.

First fruit has reached typical size (BBCH 71) with a significant delay of 12-16.75 days in the case of the variant without mulch.

The beginning of fruit ripening (BBCH81-BBCH88) was significantly delayed in the no-mulch variant, even by a month compared to the variant in which black mulching film was used.

The very large differences between the unmulched and the mulched variant had several causes. The very high temperatures in the summer of 2024 led to faster evaporation of water from soil in the case of the variant

without mulch, and the amounts of precipitation and the watering norms applied were not sufficient for an optimal supply of water to the plant. It is known that pepper is a plant with high requirements for soil water content (Munteanu, 2003). Table 1 shows that during June-July, maximum temperatures were very high, which led to the loss of a large number of flowers. In addition, the lack of water caused by the pedological drought led to an inadequate supply of nutrients to plants, which contributed to the delay in fruit growth and ripening.

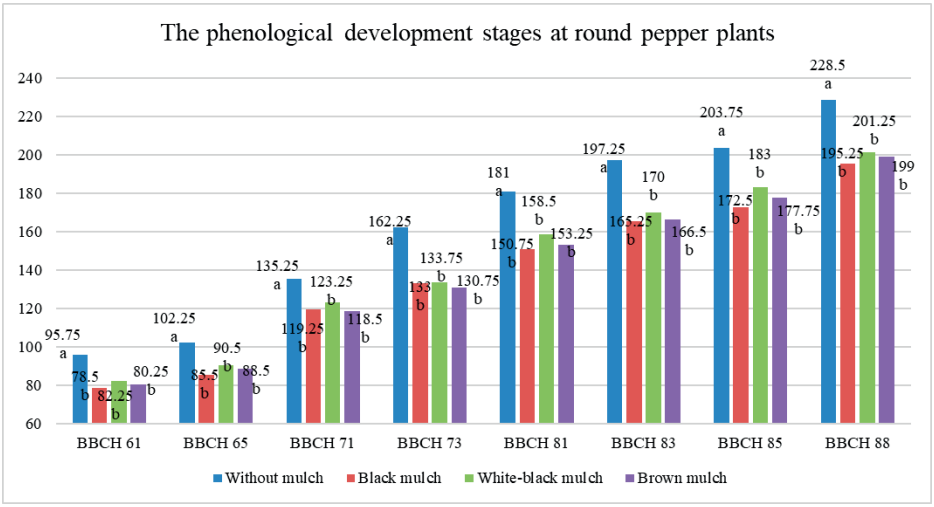


Figure 1. The influence of experimental variants the phenological development stages at round pepper

Even though Asteroid 204 is a variety with good tolerance to abiotic stress factors (Sbîrciog et al., 2016), the phenological development stages were longer due to climatic conditions, and therefore, plant development was slower. Basically, when in some mulched variants the round pepper plants were in the BBCH 85-88 development stage, the unmulched variant was still in the BBCH 73 development stage (Figure 2).



Figure 2. Round pepper plants on white-black mulching film (left) and on unmulched ground (right), 24th September

Due to the large differences in fruit ripening, three harvests were made for the mulched variants, and just two harvests for the unmulched variant. Round pepper fruits were harvested on 5th September, 19th September and 3rd October for the mulched variants, and on 19th September and 3rd October, for the variant without mulch.

In Figure 3 it can be seen which was the influence of the experimental variants on the phenological development stages at eggplant.

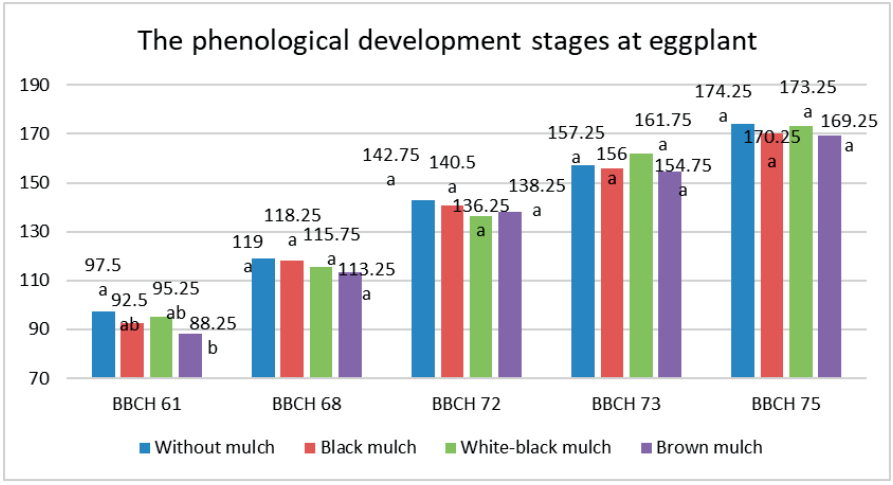


Figure 3. The influence of experimental variants the phenological development stages at eggplant

In general, eggplant is a specie that tolerates better then pepper drought and heat stress. The variety taken into the research tolerated the climatic conditions well and there were no significant differences between phenological development stages even in the case of the variant without mulch.

At eggplant, flowering took place between 14th-24th June. The brown mulch film showed a significant precocity of 9.25 days compared to the version without mulch. Even though the version without foil was much less productive, were not other delays in phenological development stages in eggplant. Fruit setting took place, without significant differences, between 15th-30th July and fruit has reached typical size and form 5th-10th August. Three harvests were carried out at 10-day intervals, starting on 5th August.

Table 2 presents the influence of experimental variants on quantity and weight of fruit, in round pepper and eggplant.

At round pepper, the number of fruits per plant was strongly influenced by the experimental variants. All mulched variants led to significant differences compared to the unmulched variant, but there were also significant differences between the mulching variants.

Table 2. The performance of peppers and eggplants under different types of mulching

Round pepper			
	Fruits per plant	Fruit weight (g)	Yield (t/ha)
Without mulch	4.18±0.23 c	135.13±12.04 a	33.88±4.13 b
Black mulch	5.09±0.15 b	151.95±19.94 a	46.55±7.27 a
White-black mulch	5.87±0.60 a	146.08±12.07 a	51.71±9.46 a
Brown mulch	5.17±0.35 b	145.95±2.18 a	45.27±3.61 a
Eggplant			
	Fruits per plant	Fruit weight (g)	Yield
Without mulch	4.53±0.43 b	210.99±30.01 b	33.10±2.02 b
Black mulch	5.00±0.35 ab	224.73±24.97 ab	39.38±5.60 ab
White-black mulch	4.93±0.41 ab	232.66±22.88 ab	40.14±5.02 ab
Brown mulch	5.19±0.32 a	251.24±12.45 a	45.65±4.52 a

*Duncan test: Mean values in a column that do not share the same letter (a, b, c) signify significant differences ($p \leq 0.05$).

The biggest number of fruits were obtained in the case of the variant in which white-blach mulching film was used, which differed significantly both from the variant without mulch, and also from the variants in which black mulching film and brown mulching film were used. The white-black mulching film led

to an average number of 5.87 fruits/plant. The black and brown mulching film led to an average number of 5.09-5.17 fruits/plant, significantly higher than the unmulched variant which produced an average of 4.18 fruits per plant.

However, the weight of round pepper fruits did not differ significantly.

The mulched variants led to significant increases of yield of round pepper, compared to the variant without mulch, but no significant differences were observed between them. The highest yield of round pepper was obtained in the case of the variant where white-black mulching film was used. The yield of 51.71 t/ha corresponds to a maximum potential of production of the Asteroid 204 variety (Sbîrciog et al., 2016). The brown and black mulching film led to a yield of 45.27-46.55 t/ha.

Basically, the plants invested water and nutrient resources in increasing the number of fruits per plant and in the fruit ripening process, but without a significant different in fruit weight.

In eggplant, the highest number of fruits per plant was obtained by using the brown mulching film. This variant led to an average number of 5.19 fruits/plant, significantly higher than the unmulched variant, which led to the formation of 4.53 fruits per plant. The other two variants also led to increases in the number of fruits compared to the variant without mulch, but the differences were insufficient to be significant.

Regarding the weight of eggplant fruits, it was significantly influenced by the variant mulched with brown film. This led to an average increase of 40.25 g compared to the variant without mulch. The increases in the case of the other two variants (black and white-blach mulching film) were insignificant.

The highest eggplant yield was obtained when the land was mulched with brown mulching film. This variant led to a yield of 45.65 t/ha, with a significant difference of 12.55 t/ha compared to the variant without mulch.

Although eggplants are known for their drought tolerance, their quality and yield can decrease under water deficit conditions. However, plant response to plastic mulch varies depending on the eggplant variety, materials used, and environmental conditions (Abdrabbo et al., 2017).

Table 3 presents the influence of experimental variants on some quality indices of round pepper and eggplant fruits.

Table 3. Comparative Analysis of Water Content, Dry Weight, Ash Content, and Total Soluble Solids in Round Pepper and Eggplant Under Different Mulching Treatments

Round pepper				
	Water (%)	DW (%)	Ash (% FW)	TSS (°Brix)
Without mulch	91.35±0.27 b	8.65±0.27 a	0.51±0.02 b	6.80±0.12 b
Black mulch	92.05±0.44 ab	7.95±0.44 ab	0.54±0.05 ab	7.30±0.27 a
White-black mulch	92.76±0.48 a	7.24±0.48 b	0.59±0.03 a	7.14±0.27 ab
Brown mulch	92.57±0.44 a	7.43±0.44 b	0.53±0.04 ab	7.17±0.26 ab
Eggplant				
	Water (%)	DW (%)	Ash (% FW)	TSS (°Brix)
Without mulch	90.80±0.60 b	9.20±0.60 a	0.66±0.10 ab	4.13±0.06 c
Black mulch	91.61±0.22 ab	8.39±0.22 ab	0.68±0.03 ab	4.54±0.27 b
White-black mulch	92.21±0.17 a	7.79±0.17 b	0.78±0.04 a	4.90±0.17 a
Brown mulch	92.30±0.63 a	7.70±0.63 b	0.59±0.04 b	4.52±0.07 b

*Duncan test: Mean values in a column that do not share the same letter (a, b, c) signify significant differences ($p \leq 0.05$).

The composition of the dry matter in fruits includes sugars, especially carbohydrates, of which glucose and fructose constitute approximately 48% of the total. In addition, lipids, proteins, minerals, vitamins, organic acids and phenolic compounds are found (Kader, 2002). In the experiment, the total dry matter was found to be highest in the variant without mulch. In contrast, all types of mulch demonstrated superior effectiveness in maintaining adequate plant hydration. Specifically, the use of black-and-white and brown foils yielded more favorable results in this regard compared to the black foil mulch for both species (Table 3). These findings align with the observations reported by Iqbal et al. (2015). Furthermore, the composition of dry matter can be influenced by various factors, including genotype, environmental conditions, cultivation practices, and available water levels (Gruda et al., 2025; Rouphael et al., 2012; Zhou et al., 2024).

The role of soluble solids, together with other phytochemicals, is essential in defining fruit aroma. The soluble solids influence the

refractive index of aqueous extracts of the fruit and among these can be included soluble sugars, soluble pectin, organic acids and various non-carbohydrate compounds (Diaconu, 2006). An analysis of the impact of mulching on the content of soluble solids is presented in Table 3. Compared to the control variant (without mulch), significant differences were observed between the type of black mulching film for round pepper and black-and-white mulching film for eggplant. Thus, for round pepper, the variant without mulch had the lowest values (4.13°Brix), while the black-and-white mulch recorded the highest values (4.90°Brix), significantly higher than the first variant. Mehraj et al. (2015) also mentioned a significantly higher influence of TSS content in plums under the influence of black polyethylene mulch. In eggplant, all types of mulch significantly influenced this quality parameter compared to unmulched soil. In this case, the highest values of this indicator were observed in black-and-white mulch (4.90°Brix) followed by black (4.54°Brix) and brown (4.52°Brix) mulch and the lowest in unmulched soil (4.13°Brix). This aspect is particularly important for fruits intended for industry, given that the profitability of the product is directly influenced by the content of soluble solids (Stevens, 1972).

The influence of soil mulching on physicochemical parameters was significant, with higher average values observed in mulched soil compared to bare soil, except for dry residue. This study suggests that plastic mulch can be an effective option for improving the quality and quantity of agricultural products. In conclusion, the use of plastic mulching can significantly improve crop yields in agriculture, especially in areas affected by moisture deficit.

The study of the effects of different types of mulching revealed that the fruits of the two species exhibited a slight increase in ash content, with significantly higher values in the presence of black-and-white mulching film compared to the no-mulch variant. The improvement of ash content was particularly observed under the influence of various types of green mulch, such as in the case of Aloe vera (Darini, 2019) or sweet potato (Kosterna, 2014). The literature shows that plastic mulch can contribute to improving the nutrient content in pepper plants (Gao et al., 2019).

When analyzing the effect of mulching practices on fruiting parameters and quality indicators in peppers, it is observed that different mulch films have a significant impact on yield per hectare and, consequently, on the number of fruits per plant (Table 4).

Table 4. Analysis of Pearson correlations between mulching practices and production characteristics or fruit quality indicators in round pepper

	Types of mulching	Fruits/ plant	Fruit weight (g)	Yield (t/ha)	Water (%)	DW (%)	TSS (°Brix)	Ash (% FW)
Types of mulching	1	0.613(*)	0.230	0.509(*)	0.457	-0.457	0.526	0.298
Fruits/ plant		1	0.508(*)	0.921(**)	0.579(*)	-0.579(*)	0.592(*)	0.624(*)
Fruit weight (g)			1	0.799(**)	0.099	-0.099	0.327	0.064
Yield (t/ha)				1	0.490	-0.490	0.600(*)	0.491
Water (%)					1	-1.000(**)	0.004	0.077
DW (%)						1	-0.004	-0.077
TSS (°Brix)							1	0.781(**)
Ash (% FW)								1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Additionally, an improvement in fruit hydration is noted in mulched plants; however, this relationship is not statistically significant. The relationship between fruit yield and their weight or the number of fruits per plant is positively correlated and statistically significant (r=0.799 and r=0.921, respectively). There is also a positive interdependent relationship, though of lesser intensity, between the number of fruits per plant and their weight, fruit water content, total soluble solids (TSS), and ash content, with correlation coefficients of

(r=0.508, r=0.579, r=0.592, and r=0.624, respectively). Furthermore, the correlation between TSS and ash content in fruits is positive and statistically significant (r=0.781).

Table 5 presents the influence of mulching practices on the production and quality indicators of eggplant fruits. The results obtained indicate a significant positive linear interdependence between mulching practices and the number of fruits per plant, as well as their weight (r=0.521, r=0.576). Additionally, there is a significant correlation between different types of mulch and yield, as well as the hydration of the biomass produced (r=0.726 and r=0.808, respectively). The correlation between the number of fruits per plant and crop yield is significant (r=0.667). The relationship between fruit weight and yield is of much higher intensity (r=0.835). The degree of correlation between the hydration status of the fruits and TSS is slightly lower (r=0.691), yet the correlation remains significant.

Table 5. Analysis of Pearson correlations between mulching practices and production characteristics or fruit quality indicators in eggplants

	Types of mulching	Fruits/ plant	Fruit weight (g)	Yield (t/ha)	Water (%)	DW (%)	TSS (°Brix)	Ash (% FW)
Types of mulching	1	0.521(*)	0.576(*)	0.726(**)	0.808(**)	-0.808(**)	0.567(*)	-0.175
Fruits/ plant		1	.149	0.667(**)	0.443	-0.443	0.421	-0.105
Fruit weight (g)			1	0.835(**)	0.195	-0.195	0.023	-0.230
Yield (t/ha)				1	0.369	-0.369	0.253	-0.197
Water (%)					1	-1.000(**)	0.691(*)	-0.114
DW (%)						1	-0.691(*)	0.114
TSS (°Brix)							1	0.430
Ash (% FW)								1

*. Correlation is significant at the 0.05 level (2-tailed).
*. Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

The use of plastic mulch significantly influenced the phenological development stages of round pepper, variety Asteroid 204, but the eggplant cultivar Belona did not show any differences in plant development.

The number of fruits per plant and the yield of round peppers was significantly influenced by all mulching variants, the highest values being obtained when mulching with black-and-white foil.

In eggplant, brown mulching film led to the highest values of the number of fruits per plant, fruit weight and yield.

The variants with white-black and brown mulching foil led to significant increases in fruit water content, both in round pepper and eggplant, meaning a better hydration of them.

The total soluble solids content in round peppers was positively influenced by the use of black mulching foil, and in eggplants, all mulching variants led to significant increases compared to the no-mulch variant.

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SCoT MARKER-BASED ANALYSIS AND BIOCHEMICAL TRAITS EVALUATION OF TOMATO VARIETIES

Mirela Irina CORDEA[1], Mihaiela CORNEA-CIPCIGAN[2], Doina CLAPA[1], Ioana MOLDOVAN[3], Lehel LUKACS[3], Rodica SIMA[1], Alexandru-Ioan APAHIDEAN[1], Rodica POP[1], Ioana POP[4], Costel VÎNĂTORU[5], Bianca MUŞAT[5], Camelia BRATU[5], Monica HÂRŢA[1]

[1]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[2]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[3]Horticulture Research Station, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 5 Horticultorilor Street, Cluj-Napoca, Romania
[4]Faculty of Forestry and Cadastre, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[5]Plant Genetic Resources Bank for Vegetable, Floriculture, Aromatic and Medicinal Plants Buzău, 56 Nicolae Bălcescu Blvd, 120246, Buzău, Romania

Corresponding author email: monica.harta@usamvcluj.ro

Abstract

This study evaluates the genetic diversity of nine tomato genotypes using SCoT (Start Codon Targeted polymorphism) molecular markers and assesses their acidity and Vitamin C levels to determine nutritional properties. Among the primers analyzed, 11 generated 118 bands, of which 78 were polymorphic, with polymorphic band percentages ranging from 60% to 80%. The average polymorphic information content (PIC) was 0.29, with the highest value for SCoT 10, while the mean diversity index (H) and marker index (MI) were 0.360 and 3.40, respectively. Hierarchical Cluster Analysis (HCA) divided the genotypes into two main groups and revealed a moderate-high level of similarity between the analysed varieties clustered in the same group. Acidity levels ranged from 0.21% in Kumato Nero cv. to 0.73% in Claudia cv., while Vitamin C content varied between 11.03 mg/100 g (Kumato Nero cv.) and 17.11 mg/100 g (Matilda cv.). Kumato Nero exhibited the lowest Vitamin C and acidity levels, suggesting high consumer acceptance. This research highlights the importance of assessing genetic diversity and also nutritional value, providing valuable insights for future breeding programs to improve tomato traits.

Key words*: Solanum lycopersicum L., acidity, vitamin C, molecular marker, tomatoes.*

INTRODUCTION

The tomato (*Lycopersicon esculentum* L.) is a widely cultivated vegetable with diverse applications worldwide. As one of the most important fruit crops globally, tomatoes are a rich source of nutrients, particularly vitamin C and essential minerals like phosphorus, iron, and calcium. Due to the rising demand for high-quality produce throughout the year, tomato cultivation in controlled environments has become increasingly popular, allowing growers to maximize productivity and quality regardless of seasonal changes. The tomato is recognized not only for its global agricultural importance but also for its nutritional profile, including significant levels of vitamin C and soluble solids that contribute to its taste, shelf life, and health benefits (Oliveira et al., 2013; Turhan et al., 2011; Dorais et al., 2008). The through assessment of biochemical cahracteristcs is a crucial component of breeding programs since these qualitative features are important for both fresh consumption and processing. In an effort to find accessions with better nutritional and organoleptic qualities, recent efforts have concentrated on assessing spontaneous variation within tomato germplasm (Jia et al., 2011; Tripodi et al., 2021; Tripodi et al., 2024). Considering its capacity to provide insight into cultivar variability and contribute to selecting

desired characteristics, genetic diversity analysis has emerged as a key component of contemporary plant breeding programs (Shahlaei et al., 2014). The capacity of Start Codon Targeted (SCoT) markers to identify polymorphisms associated with functional genes, as well as their cost-effectiveness and repeatability, have made them renowned amongst the multitude of the molecular markers that are now readily available (Collard and Mackill, 2008; El-Fiki et al., 2021). By effectively defining genetic diversity in tomato accessions, studies using SCoT markers have paved the road for precision breeding techniques that combine molecular information with phenotypic quality metrics (El-Fiki et al., 2021; Abdein et al., 2018; EL-Mansy, et al., 2021).

Furthermore, a through method of tomato advancement is provided by the evaluation of both genetic variation and quality attributes such as soluble solids and vitamin C content. It is well known that soluble solids, which signify sugars and specific metabolites brought on by stress, can change depending on environmental and genetiv variables (Jia et al., 2011) Comparably, the vitamin C level of tomato fruits plays a crucial role in their antioxidant defence system, impacting not just human health, but also their shelf life and postharvest quality (Constantino et al., 2022). The interplay between these traits highlights the potential of integrating biochemical characterization with molecular marker-assisted selection; a strategy increasingly advocated accelerating the breeding process (Jia et al., 2011; Constantino et al., 2022).

In light of the above, this study focuses on a detailed genetic diversity analysis of tomato varieties using SCoT markers while concurrently evaluating soluble solids, titratable acidity and vitamin C content. This approach is anticipated to facilitate the identification of genetically diverse and nutritionally superior tomato varieties, reinforcing breeding programs aimed at developing cultivars with enhanced quality characteristics and environmental adaptability (El-Fiki et al., 2021; Constantino et al., 2022). The integration of these methodologies not only strengthens the understanding of the genetic basis underlying quality traits but also promises to streamline the selection process in tomato breeding programs, ensuring that high nutraceutical value and consumer preferences are met.

MATERIALS AND METHODS

Plant material

The experiment was carried out in the greenhouse of the Horticultural Research Station UASVM Cluj-Napoca. The biological material was represented by nine tomato genotypes from Buzău Plant Genetic Resources Bank: Matilda (T1), Măriuca (T2), Kumato Nero (T3), Claudia (T4), Vitamina (T5), Serena (T6), Siriana (T7), Kristinica (T8) and Darsirius (T9).

DNA extraction

DNA was isolated from the tomato leaves using the CTAB (Cetyltrimethylammonium bromide) technique, based on the procedure published by Lodhi et al. (1994). The extracted DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to ascertain the amount and purity of each evaluated sample.

Genetic analyses using SCoT markers

The molecular markers SCoT were employed in the present investigation as based on the amplification of conservative and particular areas flanking the start codon (ATG) of the methionine amino acid on both sides (Table 1). The components of the PCR reaction mixes were as follows L Green Master Mix (Promega), 20 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl, 0.24 mM dNTPs, Taq polymerase (0.5 U) (Promega), primer (0.8 µm), and template DNA (25 ng). With minor adjustments, amplification was carried out according to the procedure outlined by Collard and Mackill (2008): denaturation for three minutes at 94°C, followed by 35 cycles of 94°C, 50°C, and 72°C for one minute each, and a final extension at 72°C for five minutes. A 1K bp DNA ladder (Bioline, Inc. USA) was used to separate the PCR amplification products. The separation was performed on agarose gels (2%) (Promega, Madison, Wisconsin, USA) in 1 x TAE, at 0.29 V/cm^2 for 1.5 hours, and detected by EtBr staining

(Sigma-Aldrich GmbH, Darmstadt, Germany). PCR amplifications were carried out in duplicate and visualized using the UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany). Segments of SRAP and SCoT that were easily reproducible were assessed as either present (1) or absent (0).

Table 1. The SCoT primer sequences used for the assessment of genetic relationships of tomato varieties

SCoT primer	Sequence (5′-3′)	Tm (°C)	GC content (%)
SCoT 1	CAACAATGGCTACCACCA	52	50
SCoT 3	CAACAATGGCTACCACCG	53.7	56
SCoT 4	CAACAATGGCTACCACCT	51.8	50
SCoT 5	CAACAATGGCTACCACGA	52	50
SCoT 6	CAACAATGGCTACCACGC	54.3	56
SCoT 9	CAACAATGGCTACCAGCA	58.3	50
SCoT 10	CAACAATGGCTACCAGCC	57.5	56
SCoT 11	AAGCAATGGCTACCACCA	62.8	50
SCoT 12	ACGACATGGCGACCAACG	59.5	61
SCoT 13	ACGACATGGCGACCATCG	58.3	61
SCoT 14	ACGACATGGCGACCACGC	62.7	67

Determination of TSS, TA and Vitamin C

Total Soluble Solids (TSS%) was determined by the index of refraction measured with a portable refractometer. Titratable Acidity (TA), expressed as % of citric acid (CA), was determined by titration of the sample with sodium hydroxide, using phenolphthalein as indicator. Vitamin C (mg/100 g) was determined by redox titration with potassium iodate in the presence of potassium iodide.

All analyses were performed in triplicate for the nine cultivars.

Statistical Analysis

The results of the study were analyzed using statistical software R4.4.2 ("Pile of Leaves") (R Core Team, 2024). The significance level was set at $p < 0.05$. The normality of the data was tested using the Shapiro-Wilk test and descriptive statistics.

The data showed normal distribution and were described as mean ± standard deviation (SD). The One-Way ANOVA test, followed by the Tukey post-hoc, was used to verify the existence of significant differences between cultivars. Pearson correlations and linear regressions were used to test for a link between the studied variables. The data were analyzed by IBM SPSS Statistics 19.0., ORIGINPRO 2021 v.9.8 and PAST 4.1.1. software.

RESULTS AND DISCUSSIONS

Genetic diversity by SCot markers

Molecular characterization of tomato varieties was evaluated using selected SCoT markers that produced clearly reproducible DNA bands (Figure 1). Among the evaluated primers, 11 generated higher polymorphism, with a total of 118 bands, out of which 78 proved to be polymorphic. The polymoprhic bands (PB) per selected primer ranged between 2 (SCoT 11) and 12 (SCoT 4 and SCoT 14). PB percentage varied between 80.0% and 60.0% with an average of 52.18%. The tested markers' polymorphisms and discriminatory ability were assessed using indicators including polymorphic information content (PIC), diversity index (H), marker index (MI) (Table 2).

Table 2. Description of the SCoT primers selected for the assessment of genetic relationships of tomato varieties

Primers	No. AB	No. PB	Polymorphism (%)	H	PIC	E	MI	D	R
SCoT 1	10	6	60	0.06	0.06	0.44	1.89	1.00	0.89
SCoT 3	12	9	75	0.30	0.26	2.78	6.21	0.97	3.78
SCoT 4	17	12	70.59	0.38	0.31	3.11	1.11	0.93	4.00
SCoT 5	10	6	60	0.33	0.27	3.11	7.58	0.96	2.67
SCoT 6	16	10	62.50	0.44	0.35	5.00	1.65	0.89	2.89
SCoT 9	5	4	80	0.33	0.27	3.11	7.58	0.96	4.00
SCoT 10	3	2	66.67	0.50	0.37	7.22	2.67	0.77	2.89
SCoT 11	10	6	60	0.33	0.23	3.22	6.53	0.91	3.22
SCoT 12	12	7	58.33	0.40	0.32	4.11	1.21	0.93	4.00
SCoT 13	8	5	62.50	0.38	0.31	3.78	1.05	0.94	1.78
SCoT 14	16	12	75	0.49	0.37	8.56	3.11	0.68	2.22
Total	119	79	-	-	-	-	-	-	-
Mean	9	5.64	52.18	0.36	0.29	4.12	3.41	0.90	2.91

Note: AB = amplified bands; PB = polymorphic bands; H = genetic diversity; PIC = polymorphic information content; E = effective multiple ratio; MI = marker index; D = diversity index; R = resolving power.

The mean value of PIC was 0.29, with the highest in SCoT 10, whereas the average values of H and Mi were 0.360 and 3.40, respectively. The HCA organized the varieties in two major groups. The primary cluster comprised Matilda (T1) and Mariuca (T2) with the highest similarity. The following cluster positioned the variety Serena (T6), which proved to be distinct from the others and closely grouped with Siriana (T7). The following sub-cluster positioned Claudia (T4) and Vitamina (T5) tomato varieties into the same group. The last sub-cluster organized variaties Darsirius (T9)

and Kumato Nero (T3) which were closely grouped with Kristiana (T8). In a different study, the genetic diversity and relationships among 64 accessions were analyzed using 26 SCoT markers which generated 294 polymorphic bands and a PIC value of 0.84 (Rasul et al., 2022). Shahlahei et al. (2014) reported that out of the used markers, 10 generated a total of 83 bands, out of which 30 proved to be polymorphic.

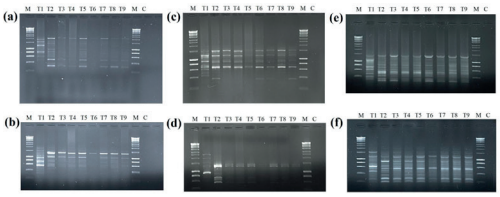


Figure 1. Amplification profiles of SCoT 4 (a), SCoT 5 (b), SCoT 6 (c), SCoT 8 (d), SCoT 12 (e), and SCoT 14 (f). Lanes T1-T9 represent different tomato varieties; M-1Kb ladder (Bioline); C- negative control

As shown in Figure 2 the HCA organized the varieties in two major groups. The primary cluster comprised Matilda (T1) and Mariuca (T2) with the highest similarity. The following cluster positioned the variety Serena (T6) which proved to be distinct from the others and closely grouped with Siriana (T7). The following sub-cluster positioned Claudia (T4) and Vitamina (T5) tomato varieties into the same group. The last sub-cluster organized varieties Darsirius (T9) and Kumato Nero (T3), which were closely grouped with Kristiana (T8).

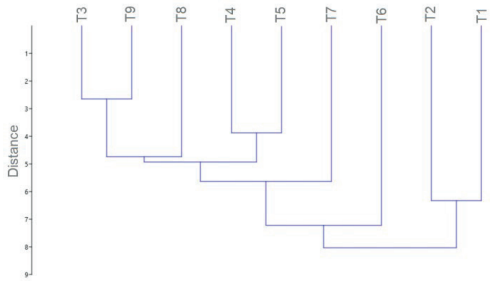


Figure 2. The HCA depicting the genetic relationship among the evaluated tomato varieties from the SCoT analysis (Euclidean distance, R = 0.84).

The genetic improvement of tomato varieties increasingly relies on an integrated approach that couples molecular marker analysis with the assessment of key fruit quality attributes such as titratable acidity, soluble solids, and vitamin C content.

Recent studies demonstrated that SCoT markers allowed for the clear differentiation of tomato accessions, validating their utility in comprehensive diversity studies (Shahlaei et al., 2014). Al-Ghufaili et al. further supported this conclusion by showing that cluster analyses based on SCoT data effectively divided tomato genotypes into distinct phylogenetic groups, providing insights into the underlying genetic relationships essential for breeding strategies (Al-Ghufaili et al., 2023). Furthermore, according to the HCA a maximum coefficient of 0.8 has been observed in tomato accessions according to their area of collection, namely AC36 (Rozh) and AC40 (Roma), respectively. These tomato accessions in the SCoT dataset may be further used in future breeding strategies (Rasul et al., 2022). In a different study, the use of SCoT markers generated 9 polymoprhic bands, with an average of 5 bands per primer and the highest polymorphism of 50% detected for SCoT 2 and 15. Furthermore, the highest PIC (0.31) and MI (0.47) has been observed in SCoT 2, whereas the highest Rp (13.1) has been detected in SCoT 12. The HCA separated the tomato accessions in two groups, with G1, G3 and G5 positioned in the same cluster (EL-Mansy et al., 2021).

Evaluation of TSS, TA and Vitamin C

In parallel with the molecular investigations, the assessment of biochemical markers, including titratable acidity, soluble solids, and vitamin C content, is essential for evaluating the nutritional and organoleptic qualities of tomato fruits. Phenotypic evaluations of these traits provide critical information regarding the fruit quality and consumer acceptability of different cultivars. Recent studies have highlighted that biochemical parameters are often correlated with underlying genetic differences. For example, research incorporating phenotypic trait analysis with molecular marker data has underscored the potential of using such an integrated approach to identify and select superior genotypes that combine both desirable fruit quality traits and robust genetic backgrounds (Radzevičius et al.,

2024). This dual approach is particularly useful for breeding programs focused on enhancing flavor, nutritional quality, and stress resilience, as it allows for the integration of molecular fingerprints obtained via SCoT markers with biochemical profiles (Radzevičius et al., 2024).
Statistically significant differences ($p<0.05\%$) were revealed in the present study among the studied cultivars in terms of acidity, vitamin C and total soluble solids content of tomato fruits (Table 3).
Total soluble solid (TSS) is one of the most important quality factors for tomato (Agbemavor et al., 2014), being represented by water-soluble components, mostly consisted of sugars (glucose, sucrose and fructose), organic acids and water-soluble proteins (pectin) (Astuti et al., 2018). Since sugars are the main constituents of total soluble solids, representing 65 to 70%, the measurement of TSS content fairly estimates the sugar level in tomato fruit Malundo et al., 1995; Saliba-Colombani et al., 2001; Agbemavor et al., 2014. The flavour of tomatoes, which is most likely to match the consumer perception of the internal quality, is dependent on both high sugar and acids content, meaning that high acids and low sugars will produce a sour tomato, while high sugars and low acids will result in a tasteless tomato (Astuti et al., 2018; DeBruyn et al., 1971). Thus, soluble solids and titratable acidity are the main components responsible for tomato flavour (DeBruyn et al., 1971).
Total soluble solids content (Table 3) showed significant statistical differences ($p<0.05\%$), ranging from 2.88% ('Darsirius') to 6.71% ('Matilda') in the fruit of the studied cultivars. Arazuri et al. (2007), in a study which evaluated fruits of some breeding lines of tomatoes, stated that total soluble solid of 4.80 to 8.80% indicates the high quality of tomato. Citric acid (CA) is the most dominant organic acid in tomatoes, which besides malic acid, are the organic acids that contribute to the flavour of tomatoes (Agbemavor et al., 2014). The titratable acidity (TA) content in tomato fruit of the studied cultivars ranged from 0.35% to 0.88%. The highest acidity values were recorded at 'Claudia' (0.73%), followed by 'Vitamina' (0.21%) and 'Darsirius' (0.68%) cultivars. The results are in line with those reported by Agbemavor et al., 2014.Tomato supply important antioxidants for health, such as vitamin C (Bui et al., 2010). The vitamin C content in the fruit of the studied cultivars ranged between 11.0 mg/100 g in 'Kumato Nero' and 17.1 mg/100 g in 'Matilda' cultivar, which were in accordance with those reported by Bui et al., 2010; Kaur et al., 2006.
Sugars, acids, and their interactions are important to sweetness, sourness, and overall flavor intensity in tomatoes (DeBruyn et al., 1971). Regarding the vitamin C and total soluble solids content, 'Matilda' cultivar showed the highest values, with an average of 17.1% vitamin C and 6.71% soluble solids, respectively (Table 3).

Table 3. Chemical characteristics of the fruit in nine tomato cultivars

Cultivars (cod)	Titrable acidity (TA) (%CA)	Vitamin C (mg/100 g)	Total Soluble Solids (TSS) (%)
Matilda (T1)	0.63 ± 0.03^{bc}	17.1 ± 0.49^{a}	6.71 ± 0.10^{a}
Măriuca (T2)	0.49 ± 0.02^{d}	12.3 ± 0.19^{c}	5.01 ± 0.10^{d}
Kumato Nero (T3)	0.21 ± 0.02^{e}	11.0 ± 0.70^{d}	3.41 ± 0.10^{e}
Claudia (T4)	0.73 ± 0.01^{a}	13.2 ± 0.65^{c}	6.11 ± 0.20^{b}
Vitamina (T5)	0.69 ± 0.03^{c}	15.1 ± 0.11^{b}	6.20 ± 0.10^{b}
Serena (T6)	0.60 ± 0.01^{c}	13.5 ± 0.49^{c}	5.70 ± 0.10^{c}
Siriana (T7)	0.62 ± 0.02^{c}	15.3 ± 0.50^{b}	5.30 ± 0.10^{d}
Kristinica (T8)	0.47 ± 0.01^{d}	12.8 ± 0.22^{c}	3.50 ± 0.10^{e}
Darsirius (T9)	0.68 ± 0.03^{ab}	13.1 ± 0.19^{c}	2.88 ± 0.12^{f}

Note: The data presented are means±S.D. The letters in common indicate no significant differences between tomato cultivars within the same fruit chemical characteristics according to Tukey's HSD test at $p<0.001$.

Among the evaluated tomato fruits, the identified biochemical traits were shown to have statistically significant outcomes (Table 4).
Tomato acidity content was strongly impacted by the presence and identified levels of vitamin C and total soluble solids. Furthermore, it has been observed that the vitamin C levels of tomato fruits was significantly impacted by the total soluble solids amount.

Table 4. The relationship between the chemical characteristics of tomato fruit in nine tomato cultivars

	Titratable Acidity (% CA)	Vitamin C (mg/100 g)	Total Soluble Solids (%)
Acidity (% Citric Acid)	-		
Vitamin C (mg %)	0.622**	-	
Soluble solids Content	0.534**	0.695***	-

Note: *p < 0.05, ** p < 0.01, *** p < 0.001.

According to the relationships between the acidity and vitamin C levels, it has been observed that the fruit's acidity and vitamin C were shown to be positively correlated. Approximately 38% of the fruit's acidity is responsible for the differences in the amount of vitamin C content (Figure 3).

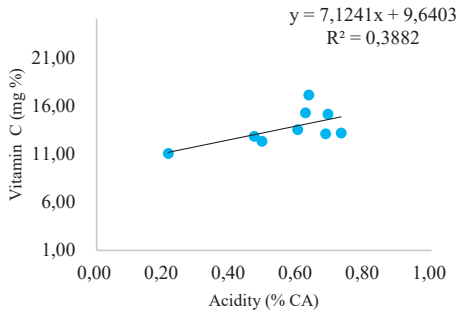


Figure 3. The association between the vitamin C content and acidity level in tomato fruits

Following, the fruit's acidity and total soluble solids content were also shown to be positively correlated. Approximately 28% of the total soluble solids prove to be responsible for the fluctuations in acidity levels (Figure 4).

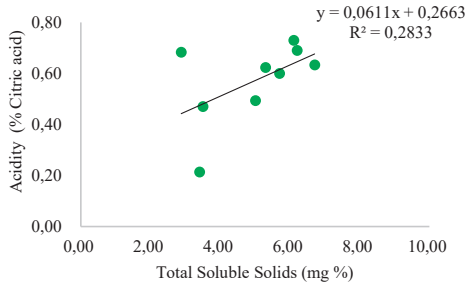


Figure 4. The association between acidity level and the vitamin C content in tomato fruit

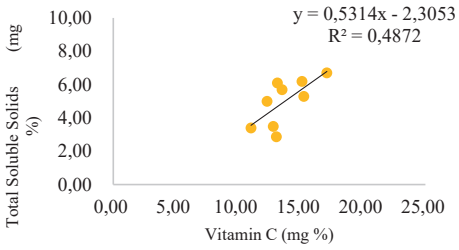


Figure 5. The association between total soluble solids and the vitamin C content in tomato fruit

Total soluble solid content and vitamin C were shonw to be positively associated, with the vitamin C concentration accounting for nearly 48% of the variance in the total soluble solids content (Figure 5).

Regarding the acidity concentration, the highest amount was identified in genotypes T4, T5 and T9, varied between 0.7-0.68% citric acid/100 g and the lowest was registered in the T3 genotype (0.21% citric acid/100 g). Results revealed significant variations among cultivars, with vitamin C content ranging from 11.03 mg/100 g to 17.11 mg/100 g. The Kumato Nero cultivar exhibited the lowest vitamin C and acidity level indicating potential for improved consumer acceptance. These results highlight the importance of cultivar selection in optimizing both nutritional quality and genetic diversity, providing valuable insights for breeding programs aimed at enhancing tomato quality.

Moreover, studies that have combined SCoT marker-based genetic analyses with evaluations of fruit quality traits have clarified that the observed genetic variability can be statistically correlated with variations in titratable acidity, soluble solids, and vitamin C (Abdeldym et al., 2020). The hypothesis that alterations in genetic profiles, as identified by SCoT markers, are complemented by quantificable alterations in fruit quality variables is partially supported by the work of El-Fiki and colleagues (2021), even though their primary focus is on generated genetic variability through irradiation and molecular marker screening. According to these results, breeding projects aiming at creating superior tomato cultivars may be more successful whether SCoT marker data and biochemical evaluations are combined for marker-assisted selection (El-Fiki et al., 2021).

CONCLUSIONS

The present investigation emphasizes how crucial it is to combine thorough phenotypic assessments of important quality attributes as soluble solids, vitamin C content, and titratable acidity with SCoT marker-based analysis for genetic diversity.

A high polymorphism ranging from 60% to 80% was obtained by 11 of the primers that were analysed, which generated 78 polymorhic bands. PIC's mean value was 0.29, and out of the evaluated primers SCoT 10 had the highest PIC value.

Acididty levels oscillated between 0.21% in Kumato Nero cultivar to 0.73% in Claudia cultivar, whilst the vitamin C content varied between 11.03 mg/100 g (Kumato Nero cv.) to 17.11 mg/100 g (Matilda cv.). Therefore, among the evaluated cultivars, Kumato Nero presented the lowest levels of vitamin C and acidity.

In addition to improving our knowledge of the genetic makeup of tomato germplasm, this all-encompassing approach makes it easier to pot for genotypes that satisfy consumer quality demands and agronomic performance requirements. The association shown in research using both molecular and chemical characteristics represents an achievable approach to improve tomato breeding initiatives and guarantee the generation of genetically viable and nutritionally superior varieties.

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CARBON FOOTPRINT OF SPINACH GROWED WITH MINERAL AND BIOLOGICAL FERTILIZATION IN GREENHOUSES

Dimitar DIMITROV, Stoyan FILIPOV, Kostadin KOSTADINOV

Agricultural University - Plovdiv, AU, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: kostadinov8888@gmail.com

Abstract

To determine the amount of greenhouse gases during the period 2021-2024, an experiment was set up with spinach under greenhouse conditions. The following options were tested: 1. spinach chamber without fertilization - control; 2. spinach chamber with mineral fertilization - NPK; 3. spinach chamber with organic fertilization - Vitaorganic; 4. Chamber without lettuce and without fertilization. At each reading, the CO2 concentration was additionally measured outdoors and in the polyethylene greenhouse. Readings were carried out weekly from planting to harvesting - from January to April. Under adverse weather conditions, young plants fertilized with organic fertilizer consume less CO2, compared to that released from the soil and available in the atmosphere. As temperatures rise, they begin to consume increasingly larger amounts, with the maximum at the end of February, after which consumption decreases to on March 28. The mineral fertilization option has a higher degree of CO2 fixation compared to organic fertilization. When entering economic maturity, the CO2 fixation values are highest for mineral fertilization, while with organic fertilization the fixation is less.

Key words*: greenhouse gases, spinach, carbon emission, mineral and biological fertilization.*

INTRODUCTION

The need for sustainable agricultural practices is increasingly urgent, particularly in addressing the environmental impact of food production. Agriculture significantly contributes to greenhouse gas (GHG) emissions, with nitrogen-based fertilizers being a major source of nitrous oxide (N_2O), a potent greenhouse gas. Agricultural production is also accompanied by the release of greenhouse gases CH_4 and CO_2 during the decomposition of plant residues. Their determination and the development of technology for their reduction have also been the subject of research (Borisova et al., 2023).

Spinach, a widely cultivated leafy green, requires intensive fertilization to achieve high yields. The carbon footprint of spinach production, particularly under different fertilization strategies, has become a key focus for researchers seeking to reduce environmental impacts while maintaining agricultural productivity (Pereira et al., 2022).

Fertilization is a double-edged sword in agriculture. While it enhances crop yields, it also contributes to significant nitrogenous gas emissions (Pan et al., 2022).

The impact of different formulations of organic and biological fertilisers on greenhouse emissions is the subject of several modern studies (Kostadinova et al., 2018; Dermendzhieva et al., 2021; Borisova et al., 2022).

The emissions from synthetic nitrogen fertilizers, particularly ammonia (NH_3) and nitrous oxide (N_2O), have been extensively documented (Xu et al., 2019; Ma et al., 2021). These gases play a critical role in global warming, air quality degradation, and ecosystem disruption. The adoption of biological fertilizers-derived from organic and microbial sources-offers a promising alternative, as they can enhance soil health and reduce GHG emissions (Yang et al., 2020).

The role of nitrogen in agriculture and its environmental implications have been the subject of extensive research. It has been highlighted the necessity of addressing nitrogenous gas emissions from croplands to transition toward low-emission agriculture (Pan et al., 2022). The authors emphasized that nitrogen management is integral to reducing the carbon footprint of agricultural systems.

Similarly, have been examined 50-year trends in nitrogen use efficiency, revealing a critical

balance between crop yield enhancement and nitrogen input optimization (Lassaletta et al., 2014). Their findings underscore the need for sustainable fertilization practices to mitigate environmental impacts.

Sustainable agricultural practices, including the use of organic and mineral fertilizers in optimal rates, are a way to reduce greenhouse gas emissions (Kuncheva et al., 2024).

Synthetic fertilisers, while effective in boosting productivity, are a primary source of ammonia volatilization (Pan et al., 2016). It is provided empirical estimates of global ammonia emissions from synthetic nitrogen fertilisers, underscoring their significant contribution to atmospheric pollution (Xu et al., 2019). Complementing these findings, refined emission factors for soil-derived ammonia, it is offered a more nuanced understanding of regional and crop-specific impacts (Ma et al., 2021).

Biological fertilizers present an eco-friendly alternative to synthetic fertilizers. It is demonstrated the efficacy of combining Azolla, a nitrogen-fixing aquatic fern, with urease inhibitors to reduce ammonia volatilization in rice cultivation, Yang et al. (2020). This strategy not only mitigates emissions but also improves nitrogen use efficiency and crop yields. It is explored the role of biochar in reducing nitrogen leaching in paddy soils, noting its potential to enhance nutrient cycling while managing GHG emissions Wang et al. (2017).

Ammonia emissions are not just a local concern but have global implications for air quality and climate. Providing insights into compliance with air quality regulations, has been analyzed the costs and benefits of ammonia emission abatement in Europe (Giannakis et al., 2019). Similarly, has been discussed how controlling ammonia emissions in China could mitigate haze pollution and nitrogen deposition, though it may exacerbate acid rain issues (Liu et al., 2019).

Nitrogen emissions are intrinsically linked to soil processes. It has been explored the relationship between redox potential, soil organic matter turnover, and nutrient cycling in submerged soils, providing a comprehensive view of nitrogen dynamics (Marschner, 2021). It is highlighted that pathways such as leaching, volatilization, and denitrification nitrogen losses from the soil-plant system have been reviewed (Cameron et al., 2013).

The interplay between carbon and nitrogen cycles is critical in understanding the environmental impacts of fertilization. The dual emissions of reactive nitrogen gases and carbon dioxide from fertilization have been examined, emphasizing the need for integrated management strategies to address these intertwined cycles (Pan et al., 2023). It has been provided a global inventory of nitric oxide emissions from soils, further elucidating the environmental footprint of agricultural practices (Davidson and Kingerlee, 1997).

The literature underscores the complexity of balancing productivity with environmental sustainability in agriculture. This study builds on these findings to compare the carbon footprint of spinach production under mineral and biological fertilization in greenhouses. By quantifying emissions and assessing mitigation strategies, the research aims to contribute to the broader goal of sustainable agriculture.

The study investigates the carbon footprint of spinach grown in greenhouses using mineral and biological fertilization, with an emphasis on understanding emissions associated with different fertilization methods.

MATERIALS AND METHODS

The experiment was conducted between 2021 and 2024 in an unheated polyethylene greenhouse at the Agricultural University of Plovdiv. The study focused on measuring greenhouse gas emissions during the autumn-winter cultivation of spinach (variety 'Matador'). The influence of organic and mineral fertilization on the CO_2 balance in the soil-plant-air system was evaluated.

Two fertilization treatments were applied: a biological fertilizer (Vitaorganic) and mineral fertilization at an active substance rate per ton of production- $N_{3.75}P_{1.25}K_{4.75}$. A control variant with no fertilization was also included.

CO_2 Emission Measurement Variants

CO_2 emissions were calculated based on the following measurement variants:

1. Control – CO_2 percentage in a chamber without fertilization and plants.

2. NPK fertilization – CO_2 percentage in a chamber fertilized with NPK but without plants.
3. Biological fertilization- CO_2 percentage in a chamber fertilized with bio-fertilizer but without plants.
4. NPK fertilization with plants – CO_2 percentage in a chamber fertilized with NPK, with plants.
5. Biological fertilization with plants – CO_2 percentage in a chamber fertilized with bio-fertilizer, with plants.
The experiment was set up with four replications. The experimental plot size was 3.36 m², while the measuring plots for determining CO_2 emissions were 0.25 m². Fertilization was applied during the final soil preparation. The organic fertilizer Vitaorganic was applied at a rate of 1500 kg/ha. Mineral fertilization was carried out using potassium sulfate, triple superphosphate, and ammonium nitrate, based on active substance rates: N – 312 kg/ha, P_2O_5 – 128 kg/ha, K_2O – 224.8 kg/ha.
Spinach was sown in early November in rows according to the scheme 70 + 6 X 15 cm, with a seeding rate of 15 kg/ha. The crop was cultivated following an established production technology for polyethylene greenhouse cultivation during the autumn-winter period (Aleksiev, 2007). Climate characteristics were recorded using a Meteobot weather station installed in the greenhouse.
To determine and visualize CO_2 emission values, three calculation models were developed:

1**. Effect of Fertilizer Type on CO_2 Emissions from Soil Microflora**
The study examined the influence of different fertilizers on CO_2 emissions from fertilized and unfertilized soils. The difference in gas emissions between the control and the fertilized variants (organic and mineral) without plants was calculated. The results are presented in Figures 1-3.
The values **X1**, displayed in the graphs, were calculated using the following formulas:

$$X1_{soil\ NPK} = C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{NPK\ fert.soil.}$$
$$X1_{soil\ BIO} = C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{BIO\ fert.soil}$$

- $X1_{soil\ NPK}$ is the displayed value of emission/absorption in camber with NPK fertilization soil in the graphs, and $X1_{soil\ BIO}$ is the value for the BIO-fertilized variant;
- $C_{\%CO_2}^{contr.soil}$ is the percentage concentration of carbon dioxide in the chamber with control soil;
-$C_{\%CO_2}^{NPK\ fert.soil}$ and $C_{\%CO_2}^{BIO\ fert.soil}$ are the percentage concentrations of carbon dioxide in the chambers with mineral (NPK) and organic fertilization, respectively.

2. **Effect of Organic and Mineral Fertilizers on CO_2 Emissions**
The data presented in Figures 4-6 were obtained by comparing chambers with plants grown in differently fertilized soils. In this case, the measured CO_2 amounts in the chamber with unfertilized soil were subtracted from the CO_2 amounts in the chambers with NPK and BIO fertilization. Positive values indicate higher CO_2 absorption compared to the chamber with unfertilized plants.
The gas exchange values for the NPK variant, as shown in the graphs, were calculated using the following formula:

$$X2_{NPK\ fert.} = C_{\%CO_2}^{non-fert.soil+plant} - C_{\%CO_2}^{NPK\ fert.soil+plant}$$

where:
- $X2_{NPK\ fert.}$ is the displayed value of NPK-fertilized in the graphs
- $C_{\%CO_2}^{non-fert.soil+plant}$ is the percentage concentration of carbon dioxide in the chamber with unfertilized soil and plants planted in it.
- $C_{\%CO_2}^{NPK\ fert.soil+plant}$ is the percentage concentration of carbon dioxide in the chamber with NPK fertilized soil and plants planted in it.

The calculations for the remaining variants were performed similarly.

3. **Influence of the fertilizers used (organic and mineral) on the emission of CO_2**
The data presented in Figures 4-6 were obtained by comparing the chambers with plants, with differently fertilized soils. In this case, the amounts of gas in the chambers from the NPK fertilization and BIO fertilization variants were subtracted from the measured amounts of carbon dioxide in the chamber with unfertilized soil. Here too, positive values mean higher absorption of CO_2 in relation to the chamber with unfertilized plants.

The values for gas exchange in the NPK variant, plotted in the graphs, were calculated according to the formulas:

$$X3_{NPK\ fert.} = (C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{NPK\ fert.+plant}) \times 10 \times 44{,}01/22{,}4,\ g/m^3$$

where:
- $X3_{NPK\ fert.}$ is the displayed value of NPK-fertilized in the graphs
- $C_{\%CO_2}^{NPK\ fert.+plant}$ is the percentage concentration of carbon dioxide in the chamber with NPK fertilized soil and plants planted in it.

The calculations for the other variants were made similarly.
Further calculations in the tables include (Table 1):

$$X_{average}^{NPK} = \left[\sum_{09.02.to\ 05.04} X3_{NPK\ fert.}\right]/9$$

$$X_{CO_2}^{NPK\ sum} = X_{average}^{NPK} \times 57\ days$$

where:
- $X_{average}^{NPK}$ is the average value of $X3_{NPK\ fert.}$ across the measured dates.
- $X_{CO_2}^{NPK\ sum}$ is the modeled CO_2 absorption for the NPK treatment over 57 days.

The calculations for the other variants are made similarly.

RESULTS AND DISCUSSIONS

The three-year study confirms that CO_2 dynamics in the soil is a complex process influenced by multiple interconnected factors, such as soil temperature, moisture, solar radiation, vegetation cover, and type of fertilization.
The graphs below present the difference in CO_2 concentration between the various experimental chambers. Positive values indicate that the soil (or plants) in the respective variant absorb CO_2, while negative values indicate emissions.

1. Influence of Fertilizer Type on CO_2 Emissions from Soil Microflora

The data presented in Figures 1-3 show that the soil temperature during the study period varied between 11°C and 19°C, with changes in depth remaining insignificant (Figure 1).

1.1. Influence of Soil Temperature on CO_2 Dynamics

The data presented in Figures 1-3 show that the soil temperature during the study period varied between 11°C and 19°C, with changes in depth remaining insignificant (Figure 1).

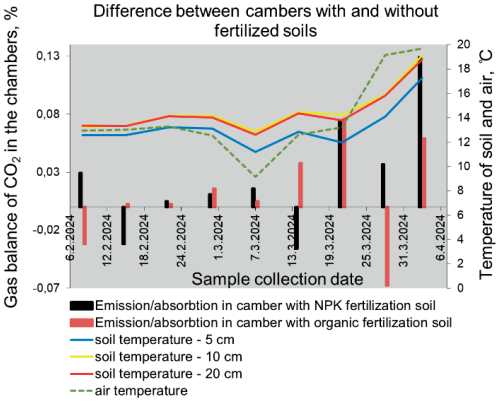


Figure 1. The temperature dependence of CO_2 dynamics in abiotic experimental chambers

The analysis of the measured CO_2 concentrations allows for an assessment of the impact of key agro-climatic factors on the soil CO_2 balance. For both fertilization methods, there is a tendency for CO_2 emissions to decrease as soil temperature rises. This highlights the importance of temperature for microbiological activity and the decomposition processes of organic matter, leading to either CO_2 emission or absorption. A particularly pronounced absorption was recorded on April 4th, when the temperature ranged between 17-19°C. Since this variant does not include plants, the increased CO_2 uptake is likely due to enhanced microbiological activity.
A comparative analysis between mineral (NPK) and biological fertilization reveals significant differences. At higher temperatures, CO_2 absorption in the NPK chamber is twice as high as in the bio-fertilized chamber. This is likely due to the high solubility and availability of mineral fertilizers, which accelerate nutrient uptake by soil microflora.
On February 8th and March 28th, CO_2 absorption was observed in the NPK variant, while biological fertilization resulted in emissions, despite minimal temperature differences (around 2°C). At the same time, on February 15th and March 14th, under similar temperature conditions, the effect was reversed-NPK led to emissions, whereas biological fertilization resulted in CO_2

absorption. This suggests that soil temperature alone is not the sole determining factor in CO_2 dynamics.

Within the temperature range of 10°C to 20°C, an inverse relationship between temperature and CO_2 emissions was established - higher temperatures lead to reduced emissions. However, during certain periods with similar temperatures, different effects were recorded, emphasizing the importance of additional factors in regulating the soil carbon balance.

1.2. Influence of Soil Moisture on CO_2 Dynamics

Soil moisture in the upper layers varied significantly throughout the experiment, ranging between 3 and 32 l/m², depending on the sampling date and soil layer depth (Figure 2). As expected, the middle and deeper soil layers (5, 10, and 20 cm) retained higher moisture levels.

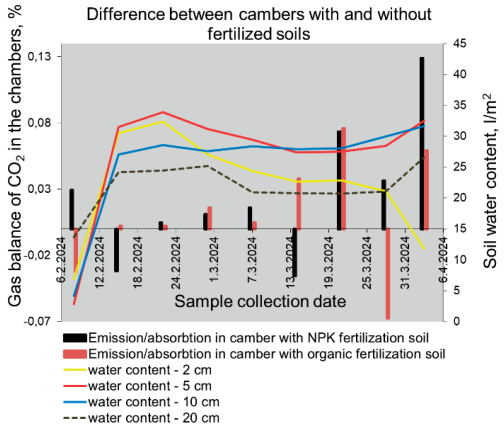


Figure 2. The effect of soil moisture on CO_2 dynamics in abiotic experimental chambers

On colder days (February 8th and March 7th), low moisture content in the upper soil layer led to increased CO_2 emissions, but only in the biologically fertilized variant. As moisture levels increased, soil permeability decreased, limiting CO_2 release. A similar trend was observed on March 28th, when moisture levels in the upper layer dropped below 21 l/m², triggering an increase in CO_2 emissions in the biologically fertilized soil.

On April 3rd, an opposite trend was observed - further reduction in soil moisture led to significant CO_2 absorption. This confirms that the CO_2 balance depends not only on individual factors but on a combination of multiple variables.

1.3. Influence of Solar Energy on CO_2 Dynamics

During the measurement period, solar energy ranged from 0.023 to 0.151 kWh/m² (Figure 3). The data indicate that at higher soil temperatures and more intense solar radiation, CO_2 absorption increases. On days with greater solar activity, such as March 21st and April 3rd, CO_2 uptake was significantly higher.

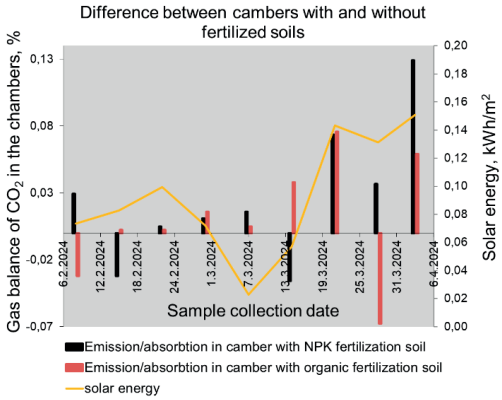


Figure 3. The radiative forcing of solar energy on CO_2 dynamics in plant-free experimental chambers

The figure illustrates that the increase in soil temperature is directly linked to the rise in solar radiation. Although the illuminated surface is small compared to the volume of the studied soil, the primary factor influencing CO_2 absorption appears to be soil temperature.

2. Influence of the applied fertilizers (organic and mineral) on CO_2 emissions

The analysis of the impact of the applied fertilizer on CO_2 levels reveals a distinct difference (Figures 4, 5, and 6). Most measurements indicate that higher amounts of CO_2 are released in chambers with mineral and organic fertilization.

This indicates that the application of mineral and organic fertilizers at the specified rates leads to increased CO_2 emissions. This effect is most pronounced during the first two measurements on February 8 and February 15, when the plants were still in their early growth stages. Soil moisture (Figure 5) does not appear to have a significant influence, as the difference

in gas emissions between mineral and organic fertilization remains consistent regardless of low or high moisture levels.

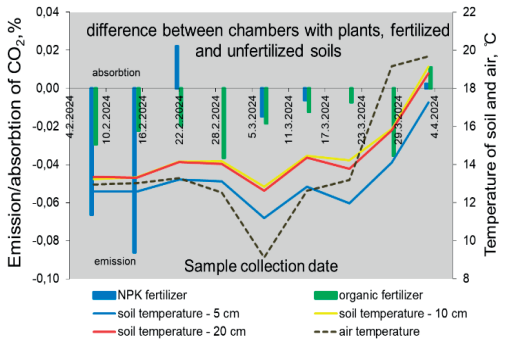


Figure 4. Influence of soil temperature on CO_2 emissions under different fertilization types

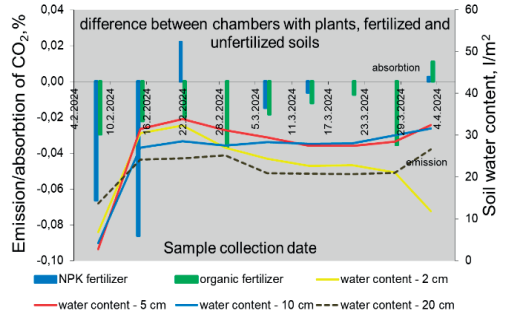


Figure 5. Influence of soil moisture on CO_2 emissions under different fertilization types

The graphs also highlight a higher CO_2 uptake in the mineral fertilization variant on February 22 and April 3. On these dates, soil moisture remained relatively stable, while temperatures varied. Among all the external factors examined, increased CO_2 absorption correlates most strongly with higher solar activity (Figure 6).

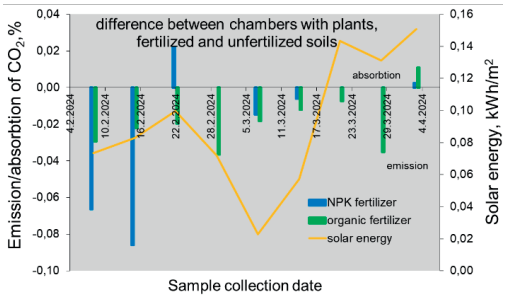


Figure 6. Influence of solar activity on CO_2 emissions under different fertilization types

The analysis suggests that at relatively lower air and soil temperatures, mineral fertilization promotes more intensive photosynthesis compared to organic fertilization. Lower temperatures slow down the mineralization of organic fertilizers, which in turn affects photosynthetic efficiency and results in less vegetative biomass formation.

Organic fertilization leads to more stable and long-term CO_2 absorption, with average values of 2.03 g/m³ and 1.89 g/m³ recorded in 2022 and 2023, respectively. However, in 2024, a decline to 1.13 g/m³ was observed, likely due to climatic anomalies.

Mineral fertilization (NPK) exhibits greater fluctuations in CO_2 absorption. While the average values were relatively high in 2022 and 2023 (1.59 g/m³ and 1.92 g/m³), they dropped to 1.18 g/m³ in 2024, suggesting a shorter-lasting effect.

Control samples (without fertilization) showed moderate and stable CO_2 absorption, though with lower overall values compared to fertilized treatments.

3. **Influence of spinach on CO_2 emissions (difference between control soil and plant variants)**

The graphs clearly illustrate that, regardless of the type of fertilizer used, CO_2 emissions are consistently lower in plant-containing variants compared to the control (unfertilized soil) in almost all measurements.

Higher air and soil temperatures, combined with increased solar activity, tend to equalize the differences between fertilized and unfertilized variants. On March 14, March 21, and April 3, the difference in CO_2 absorption was negligible.

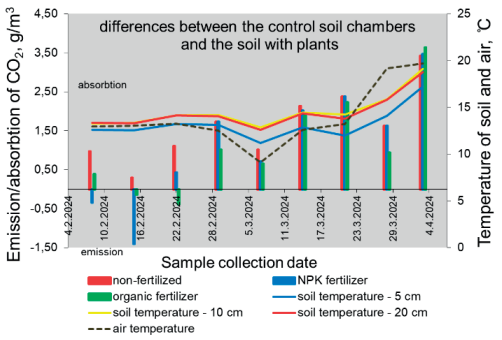


Figure 7. Influence of air and soil temperature on CO_2 emissions during plant cultivation

A temperature drop and increased cloud cover on March 7 affected photosynthesis, leading to lower carbon absorption. The decrease in air temperature was around 3°C, indicating that the primary factor behind the reduced absorption was lower solar radiation. A similar effect was observed on March 28, where despite higher temperatures, reduced solar activity resulted in lower CO_2 absorption.

An increase in soil moisture, combined with mineral fertilization during the early growth phase, led to higher carbon emissions (Figure 8).

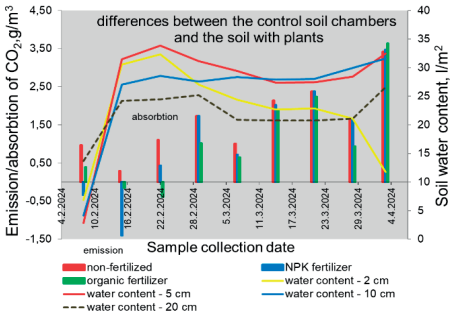


Figure 8. Influence of soil water content on CO_2 emissions during plant cultivation

This confirms the earlier observation that mineral fertilizers, when applied in the specified amounts and under conditions of relatively higher soil moisture, stimulate soil microbiota activity, potentially increasing greenhouse gas emissions.

The data show that high soil moisture correlates with increased CO_2 emissions, especially under organic fertilization. This can be attributed to the accelerated microbial decomposition of organic matter. Under optimal moisture conditions, the best balance between emissions and absorption is observed.

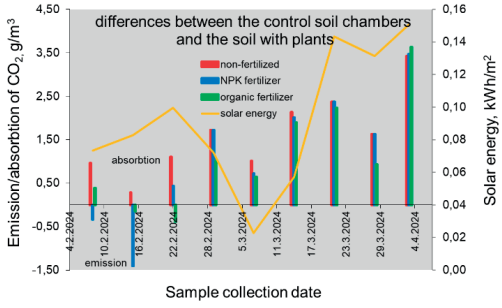


Figure 9. Influence of solar activity on CO_2 emissions during plant cultivation

4. Evaluation and Comparison of Carbon Emissions Under Different Fertilization Regimes in Winter Spinach Cultivation

Tables 1-3 compare CO_2 absorption results for similar cultivation periods of spinach over the three-year experiment. The values in the tables are calculated by subtracting the CO_2 amounts measured in the chambers with plants (for the unfertilized, NPK, and BIO variants) from those in the control soil chamber. The differrences were recorded by the chromatograph in percentage terms and converted to g/m³.

Table 1. Total amount of CO_2 absorbed during the cultivation period, g/m³, 2022

2022/57 days sample collection date	Soil temperature - 10 cm	Non-fertilized	NPK fertilizer	Organic fertilizer
9.2.2022	5.9	-0.18	0.61	0.73
14.2.2022	5.8	0.21	0.04	0.33
23.2.2022	9.9	1.66	1.71	2.02
2.3.2022	8.2	0.94	0.99	1.30
9.3.2022	7.8	0.87	0.92	1.23
16.3.2022	8.8	1.18	1.23	1.54
23.3.2022	11.8	3.25	3.30	3.61
30.3.2022	16.8	2.68	2.15	3.33
5.4.2022	15.9	3.72	3.33	4.15
average value		1.59	1.59	2.03
Total amount of CO2 absorbed during the cultivation period		90.77	90.41	115.46

Table 2. Total amount of CO_2 absorbed during the cultivation period, g/m³, 2023

2023/56 days sample collection date	Soil temperature - 10 cm,°C	Non-fertilized	NPK fertilizer	Organic fertilizer
12.1.2023	8.4	1.64	1.64	1,64
18.1.2023	8.0	2.12	2.12	2,12
25.1.2023	6.7	1.54	1.54	1,54
1.2.2023	6.7	1.94	1.94	1,94
8.2.2023	5.1	2.09	2.09	2,16
15.2.2023	8.3	2.11	2.14	2,28
22.2.2023	12.3	1.30	1.63	1,25
1.3.2023	10.3	1.39	1.53	1,25
8.3.2023	10.6	2.52	2.67	2,81
average value		1.85	1.92	1.89
Total amount of CO2 absorbed during the cultivation period		103.63	107.65	105.71

To approximate the absorption difference between the variants, an assumption was made that plants absorb the average value of all measurements taken on the respective dates over 24 hours. This average value was then

multiplied by the number of days in the study period, yielding the total amount of gas absorbed during the cultivation cycle. The study period was 57 days in 2022 and 56 days in both 2023 and 2024.

Table 3. Total amount of CO_2 absorbed during the cultivation period, g/m^3, 2024

2024/56 days sample collection date	Soil temperature - 10 cm, °C	Non-fertilized	NPK fertilizer	Organic fertilizer
8.2.2024	13.3	0.96	-0.34	0,39
15.2.2024	13.3	0.29	-1.40	-0,14
22.2.2024	14.2	1.10	0.43	-0,39
29.2.2024	14.2	1.73	1.73	1,01
7.3.2024	12.9	1.01	0.72	0,65
14.3.2024	14.5	2.13	2.01	1,89
21.3.2024	14.2	2.37	2.37	2,23
28.3.2024	15.9	1.63	1.63	0,94
3.4.2024	19.1	3.42	3.47	3,64
average value		1.63	1.18	1.13
total amount of CO2 absorbed during the cultivation period		91.13	66.10	63.53

Organic fertilization resulted in more stable and long-term CO_2 absorption, with average values of 2.03 g/m³ in 2022 and 1.89 g/m³ in 2023. However, a decline was observed in 2024 (1.13 g/m³), likely due to climatic anomalies.

Mineral fertilization (NPK) exhibited greater fluctuations in absorption. While the average values were relatively high in 2022 and 2023 (1.59 g/m³ and 1.92 g/m³, respectively), they dropped to 1.18 g/m³ in 2024, suggesting a shorter-term effect. Control samples (without fertilization) demonstrated moderate and stable CO_2 absorption but with lower final values compared to the fertilized variants.

Throughout all the experimental years, a positive correlation was observed between soil temperature and CO_2 absorption levels (Table 1). The highest absorption values were recorded at temperatures above 14°C (e.g., April 3, 2024 - 19.1°C, with a maximum absorption of 3.64 g/m³ under organic fertilization). At lower temperatures (<8°C), a reduced CO_2 exchange activity was observed, likely due to slowed microbial activity.

In 2022, the lowest emissions were observed in the variant fertilized with biological fertilizer (Table 1). This is explained by the relatively lower air and soil temperatures at the beginning of the vegetation period compared to the end of the vegetation period.

For 2023, the lowest emissions were observed in the NPK-fertilized variant, and in 2024, in the unfertilized spinach variant.

Table 4. Average absorption values of the three years (2022-2024), g/m^3

Average absorption values of the three years, g/m^3		
non-fertilized	NPK fertilizer	organic fertilizer
95.17	88.05	94.90

Soil temperature is a key factor for the dynamics of CO_2 exchange – the highest absorption is observed at temperatures above 14°C. Soil moisture affects the intensity of CO_2 emissions, with excessive moisture potentially leading to increased CO_2 release. Solar radiation has a positive effect on CO_2 absorption, particularly in the presence of plants. Organic fertilization provides a more sustainable carbon balance in the soil in the long term, while mineral fertilization has a stronger but short-lived effect. Annual variations show that climatic conditions have a significant impact on CO_2 absorption, highlighting the need for adaptive agricultural practices.

These results contribute to the understanding of the mechanisms regulating carbon balance in agroecosystems and can be used to optimize microclimate management and root nutrition to reduce CO_2 emissions and increase carbon storage in the soil.

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RESEARCH ON WEED CONTROL IN ONION AND GARLIC CROPS

Marian DOBRE

University of Craiova, Faculty of Agronomy, 19 Libertății Street, Craiova, Romania

Corresponding author email: mariandvpx@yahoo.com

Abstract

Onion and garlic crops are weeded by a wide range of weed species, including annual and perennial monocotyledonous and dicotyledonous. The application of herbicides is an economical, rapid measure, which allows the cultivation of large areas and the achievement of high production yields and increased economic efficiency. Three active substances with pre-emergence application (metolachlor, pendimethalin and oxyfluorfen) and various concentrations of oxyfluorfen with post-emergence application were tested. The results showed good control of annual monocotyledons with metolachlor and good control of annual dicotyledons with small seeds, with oxyfluorfen and pendimethalin. The post-emergence application of oxyfluorfen with various concentrations controlled annual cotyledons with large seeds (Xanthium sp. *and Abutilon theophrasti) but did not control perennial dicotyledonous species.*

Key words*: onion crop, garlic crop, herbicides.*

INTRODUCTION

In 2022, Romania was the 9th largest onion producer in the European Union and 62nd globally, with an area of 1,5670 hectares allocated to this crop, 6th in the European Union, after the Netherlands, Spain, Poland, France and Germany, and 42nd in the world. Productivity in Romania was 9,283.3 kg/ha, 24th in the European Union and 119th worldwide, half the global average of 18,536.5 kg/ha.(https://ro.wikipedia.org/wiki/Lista_țărilor_producătoare_de_ceapă).

In 2022, Romania cultivated garlic on an area of 5,200 hectares, the 2nd largest in the European Union, after Spain, and the 22nd in the world, being the 3rd producer in the European Union, after Spain and Italy, and the 33rd globally. Productivity in Romania was 4,326.9 kg/ha, 13th in the European Union and 81st worldwide, almost a quarter of the global average of 17,534.7 kg/ha. (https://ro.wikipedia.org/wiki/Lista_țărilor_producătoare_de_usturoi).

The herbicidal active ingredient of commercial herbicides and its transformations

All herbicide active ingredients contain an acidic group. In this state, the herbicide has an effect on the plant. When formulating the commercial substance, the herbicide that we buy, the active substance is chemically transformed by the manufacturer into an esterified form or in the form of a salt, by neutralizing the acidic group with an alcohol or a base. Depending on the resulting molecule, smaller or larger, the concentration of the active substance differs. When two commercial products with different active substances must be mixed in the machine tank, to be applied together, quantities of the active substance or quantities of the acidic part of the active substance are given. This substance is written on the product label with the chemical name or common name or both. The label also lists the chemically inert substance or substances present in the respective herbicide formulation. The acidic part of the active substance is the part of the molecule that is responsible for the herbicidal effect of the respective active substance. The molecule of the active substance formulated as an ester or salt penetrates the plant more easily, is more chemically stable and more easily crosses the waxy cuticle of the plant. This allows the herbicide to mix easily with water, with adjuvants, which increases the ability of the active substance to be absorbed and transported by the plant. Once inside the plant, the active substance loses the salt or ester, through enzymatic activity, the parent acidic part remains independent and manifests its herbicidal effect. So, after formulation as a salt or ester, the active substance will contain the parent acid moiety, responsible for the

herbicidal effect, and the salt or ester added to the active substance for the benefits mentioned above. The mass of the active substance molecule can therefore vary depending on the salt or ester added. This does not improve the performance of the active substance because, after the loss of the salt or ester, the same parent acid moiety will act as herbicide. This is called the acid equivalent of the active substance, i.e. the parent acid moiety, regardless of which molecule is added (Dobre M., 2019).

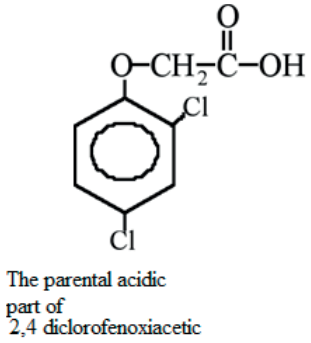


Figure 1. The parental acidic part of the active ingredient 2,4 dicolorophenoxiacid (Dobre M., 2019)

This can react with an alcohol, a base or ammonia, forming the commercial active substance, which is formulated as an ester, sodium salt or ammonium salt.

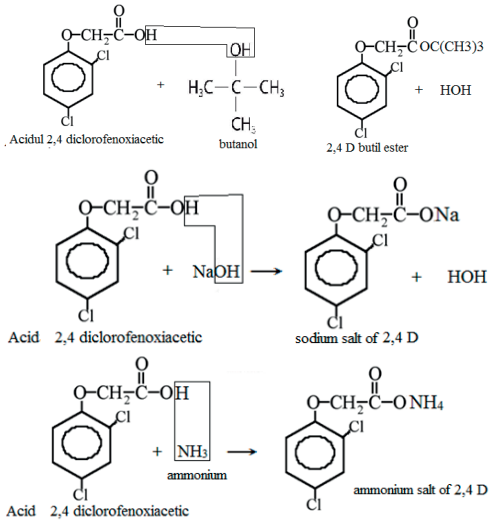


Figure 2. The active herbicidal ingredients after neutralization by an alcohol, base or ammonium (Dobre M., 2019)

Absorption of herbicides through leaves

Before exerting its herbicidal effect, any substance must first enter the leaf. To do this, it must penetrate the leaf cuticle, which is the skin that covers the leaves. This is a covering that is not made up of living cells but is composed of a layer of hydrophobic and lipophilic wax, cutin and pectin which are hydrophilic. The cuticle can be likened to a sponge where the ribs are made up of cutin and what is between them is wax. On the surface there is a layer of epicuticular wax. The cuticle differs greatly with the plant species. The absorption of herbicides in the plant is usually a passive phenomenon, based on diffusion, however, for certain active substances biochemically close to metabolic molecules, the absorption is done actively, with energy consumption, because the plant recognizes these substances as being close to those used in its metabolism. These substances are 2,4 D acid, recognized as an auxin, a growth hormone, glyphosate, recognized by the phosphate group (glyc comes from glycine, an amino acid, and phosate from phosphonate) and paraquat, recognized by putrescein, an amino acid derivative. Lipophilic herbicides are absorbed through the cuticular wax, passing easily through the wax with which the cuticle is impregnated, wax that is located between the cutin and pectin veins. Over time, the cuticle becomes more hydrophilic and the movement of lipophilic herbicides slows down. The initial absorption into the cuticular wax can represent a large percentage of the amount of lipophilic herbicide absorbed by the leaf. Furthermore, lipophilic herbicides may have difficulty passing through the cuticle into the epidermal cell layer of the leaf. A certain amount of these lipophilic herbicides can be retained by the cytoplasmic membrane (formed by two lipid layers with a double character, hydrophilic and lipophilic). For example, the herbicide Fusilade, formulated as an ester, is quickly absorbed by the cuticle. Once inside the plant, the active substance returns to its original components: the acid part, the true herbicidal component, and the alcohol. Formulating herbicides as esters is an advantage from this point of view, because they are absorbed more quickly through the wax layer of the cuticle.

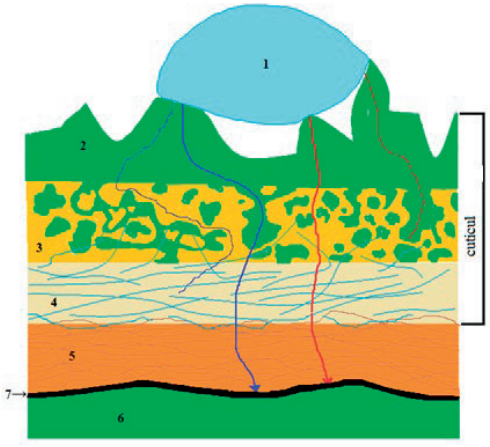


Figure 3. Differential penetration of hydrophilic (polar) and lipophilic (non-polar) herbicides through the leaf cuticle and cell wall, to the cytoplasmic membrane: 1 - spray droplet on the leaf; 2 - wax layer; 3 - cutin; 4 - pectin; 5 - cellulose cell wall; 6 - cell cytoplasm; 7 - cytoplasmic membrane

Hydrophilic herbicides, on the other hand, are more difficult to absorb through the cuticular layer, but their absorption can be improved by adding surfactants or liquid fertilizers to dissolve the wax on the leaf surface and to increase the time the spray droplet remains liquid on the leaf. Hydrophilic herbicides are absorbed through cutin and pectin, not through the cuticular wax. The absorption of these herbicides increases if they touch the pectin layer, hydrophilic, or the cell wall of the leaf epidermis. The next barrier, for some hydrophilic herbicides, can even be the cytoplasmic membrane due to its dual character, hydrophilic and lipophilic. In particular, herbicides that have the COOH group that gives the weak acidic character of the active substance, go from hydrophilic to lipophilic, and in this way, pass more easily through the cytoplasmic membrane. This phenomenon is called "ion capture" or "acid capture" and occurs depending on pH, especially in the case of weakly acidic active substances, which have a carboxylic group. The phenomenon occurs when UAN (ammonium nitrate mixed with urea – urea ammonium nitrate) or ammonium sulfate (AS) is added to the spray solution of hydrophilic herbicides, in solution form. Thus, the ammonium ion, NH_4^+, (which is actively absorbed, with energy consumption in the cell from the cell wall, through the cytoplasmic membrane) increases its concentration in the cytoplasm. Here, it separates into ammonia, NH_3, and a hydrogen ion, H^+. These ions lower the pH of the cytoplasm, but since it must remain between 7.5 and 8 units, the cell removes the excess hydrogen ions from the cytoplasm and pushes them into the cell wall, where the herbicide is. Under these conditions, the pH of the cell wall can even reach 4.5. Under conditions of high acidity, part of the herbicide passes from the hydrophilic to the lipophilic form, which we have shown above (http://www.ewrs.org/et/docs/herbicide_interaction.pdf)

An example of a hydrophilic herbicide is the well-known glyphosate which, although highly soluble in water, easily passes through the cuticle, being absorbed into the leaf up to 80%. This is possible, on the one hand, due to the hydrophilic paths of the cuticle and the use of surfactants. Also, environmental conditions can have a great impact on the absorption of hydrophilic herbicides, compared to lipophilic ones. The water content of the cuticle is lower in conditions of low relative air humidity or in drought conditions, which causes the waxy portions of the cuticle to be closer together, therefore, reducing the areas of cutin and pectin, which are hydrophilic. This determines a weaker absorption of hydrophilic herbicides (Dobre M., 2019).

The parameters pKa and lg Kow and their importance in the translocation of herbicides in the plant

The three barriers that herbicides applied to leaves must pass through are: the cuticle, the cell wall and the cytoplasmic membrane. After passing the cuticle barrier, the herbicide must enter the cytoplasm of the cell to have its effect. The state in which the active substance is in the cell wall, next to the cytoplasmic membrane is very important in the perspective of passing through it. In this position, the herbicide reaches the point where the polar (hydrophilic) and non-polar (lipophilic) forms reach equilibrium. This means that half of the molecules dissociate, giving rise to positive and negative ions and the other half remains undissociated, remaining in the lipophilic, non-polar state. This situation occurs at a certain pH, called pKa. If this pH point is within physiological limits, i.e. between 3.5 and 5.5,

then translocation through living cells, the plant's phloem, occurs and is enhanced by the ion capture phenomenon described above. In summary, this phenomenon consists of increasing the acidity of the cytoplasm by migrating acid ions and "pumping" them back into the cell wall because the pH of the cytoplasm must remain alkaline, between 7.5 and 8.0. The more acidic environment produced in the cell wall, where the herbicide is found, determines the passage of a larger amount into the lipophilic, non-polar, non-ionic form, which more easily penetrates the cytoplasmic membrane. The alkaline environment of the cytoplasm transforms the active substance, again, into the acidic, hydrophilic form, the form in which it is metabolically active, manifesting its herbicidal effect. This phenomenon occurs, in particular, in the case of active substances with the carboxylic acid group, COOH, which determines a weaker acidity. Another approach to polar, hydrophilic and non-polar, lipophilic forms is through lg Kow. This parameter represents the ratio between these two forms, that is, between the octanol-soluble form and the water-soluble form, o comes from octanol and w from water. If the ratio between the lipophilic and hydrophilic forms is 10/1, it means that for every 10 lipophilic molecules there is only one hydrophilic one. To make the expression easier, the logarithm function was used. This represents the power to which the base must be raised to give the number from the logarithm. For example, the decimal log (lg) of 10/1 is 1, that is, the power to which the base (10) must be raised to give 10/1. In the same way, the log of 100/1 is equal to 2 and the log of 100,000/1=105, that is, it is equal to 5. If the hydrophilic form predominates, the ratio between them will be 1/10 and the log of 1/10 (10^{-1}) will be -1, and so on. If the two forms are in equilibrium, the ratio will be 1/1=1. The log of 1 is zero, that is, the power to which the base (10) must be raised to give 1 (any number to the power of zero is equal to 1, thus, 100=1). The lower this ratio, the more soluble in water the active substance is, therefore, the more hydrophilic. This influences the penetration of the cytoplasmic membrane, which is a lyophilic double layer on the inside. Active substances with a high lg Kow ratio are more lipophilic and penetrate the cytoplasmic membrane more easily, penetrating the cell more easily. This parameter varies between -1 and 5. Active substances with lg Kow between -1 and 1 are more hydrophilic and penetrate the cell more difficultly. Those with lg Kow between 1 and 3 penetrate more easily, those with lg Kow = 4 are more lipophilic and part of the active substance returns to the cell wall and those with lg Kow = 5 remain trapped inside the cytoplasmic membrane, between its internal lipophilic layers because they are very lipophilic, non-polar.

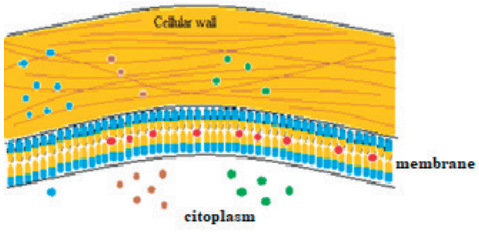


Figure 4. Penetration of herbicide active substances through the cytoplasmic membrane into the cell, depending on the lg Kow parameter

This is very important because active substances with high lg Kow, close to 5, remain trapped in the membrane, do not translocate and only affect the tissues where they entered.

Table 1. The lg Kow and pKa values for some herbicide active ingredients

Active ingredient	Lg Kow	pKa
Aminopiralid	- 2.87	2.56 (high acid)
Azimsulfuron	-1.40	3.6 (low acid)
Bentazon	-0.45	3.28 (low acid)
Bromoxinil	2.70	3.86 (low acid)
Cletodim	4.21	4.47 (low acid)
Clopiralid	-2.63	2.01 (high acid)
Dicamba	-1.88	1.77 (high acid)
Dimetenamid	2.20	Does not dissociate
Etofumesat	2.70	Does not dissociate
Flumioxazin	2.55	Does not dissociate
Fluroxipir	0.04	2.94 (strong acid)
Glifosat	-3.20	2.34 (strong acid)
Glufosinat	-3.96	2.0 (strong acid)
Imazamox	5.36	2.3 (strong acid)
Isoxaflutol	2.32	Does not dissociate
Linuron	3.00	Does not dissociate
MCPA (Mono Cloro Phenoxi Acid)	-0.81	3.73 (weak acid)
2,4 D	-0.82	3.40 (strong acid)
Metribuzin	1.65	0.99 (very strong acid)
Metolaclor	3.40	Does not dissociate
Napropamid	3.30	Does not dissociate
Nicosulfuron	0.61	4.78 (weak acid)
Oxifluorfen	4.86	Does not dissociate
Pendimetalin	5.20	2.80 (strong acid)
Rimsulfuron	-1.46	4.00 (weak acid)

MATERIALS AND METHODS

The presentation of experimental conditions and the experimental purpose

Onion and garlic crops are weeded by a wide range of weed species, including annual and perennial monocotyledons and dicotyledons. In general, the weeding work applied to combat them is costly and the labor force is expensive and increasingly scarce. The application of herbicides is an economical, fast measure, which allows the cultivation of large areas and the achievement of high production yields and increased economic efficiency. It is known that onions are resistant even to high doses of oxyfluorfen, however, the herbicide leaflet does not provide accurate information on the concentration of the herbicide in water when used on vegetation for direct-sown onions, in the first phases, nor for garlic, on vegetation. Being a contact herbicide, in high concentration it can affect young direct-sown onion plants or garlic plants. Therefore, the aim of the experiment was to find a suitable concentration that would destroy weeds in the early vegetation phase, cotyledon or the first true leaves, but would not affect the crop plants.

The experiment was located at the Botanical Garden of the University of Craiova, at the Economic Sector. Three treatments were applied for direct-sown onion, onion planted from bulbs and garlic in three replications:

- V1 - Dual Gold (metolachlor) 1.2 liters/ha in 300 liters of water applied to the sown onion;
- V2 - Pendisol 40 SC (pendimethalin) 6 liters/ha in 300 liters of water for the sown onion;
- V3 - Galigan 240 EC (oxyfluorfen) 1 liter/ha in 300 liters of water for the sown onion;
- V4 - Dual Gold (metolachlor) 1.2 liters/ha in 300 liters of water for the onion planted from bulbs;
- V5 - Pendisol 40 SC (pendimethalin) 6 liters/ha in 300 liters of water for onion planted from bulbs;
- V6 EC (oxyfluorfen) 2 liters/ha in 300 liters of water for onion planted from bulbs;
- V7 - Dual Gold (metolachlor) 1.2 liters/ha in 300 liters of water applied to garlic;
- V8 - Pendisol 40 SC (pendimethalin) 6 liters/ha in 300 liters of water applied to garlic;
- V9 - Galigan 240 EC(oxyfluorfen) 2 liters/ha in 300 liters of water applied to garlic;
- V10 - untreated control.

The experiment had 3 x 10 m long furrows that were cultivated with sown onion, planted onion and garlic. Each 10 m furrow was divided into 10 plots of 1 m length. 4 rows were sown per furrow, 25 cm apart between rows. The surface of an experimental plot was 1 m long and 1 m wide = 1 sq m. On each plot, the calculated amount of herbicide + water mixture was applied, i.e. 100 ml of solution, applied to the three plots. These substances were applied to the soil. After the emergence of crops and weeds, 4 different concentrations of Galigan 240 EC in water were applied perpendicular to the three furrows (replications).

V1 = 33 ml Galigan 240 EC in 5 liters of water (2,000 ml in 300 liters of water/ha or 0.6%);

V2 = 7 ml Galigan 240 EC in 5 liters of water (420 ml in 300 liters of water/ha or 0.14%);

V3 = 5 ml Galigan 240 EC in 5 liters of water (300 ml in 300 liters of water/ha or 0.1%);

V4 = 5 ml Galigan 240 EC in 7 liters of water (215 ml in 300 liters of water/ha or 0.07%);

V5 = untreated control.

In order to control *Sorghum halepense* and *Cynodon dactylon*, Agil 1.2 l/ha (propaquizafop) was subsequently applied when these weeds reached approximately 15 cm in height, after these species had resumed growth, following treatment with oxyfluorfen which partially necrotized them.

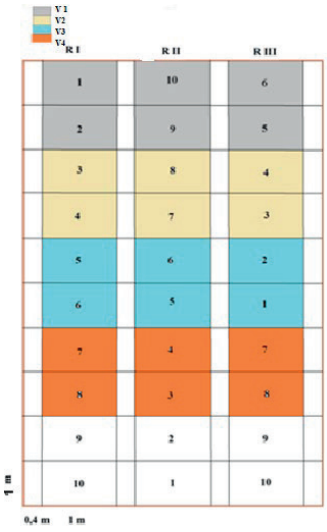


Figure 5. The experiment sketch

RESULTS AND DISCUSSIONS

a. **In the case of herbicides applied to the soil**

The results of the efficacy of the herbicides Dual Gold (metolachlor), Pendisol 40 SC (pendimethalin) and Galigan 240 EC (oxyfluorfen) as well as the phytotoxicity on sown onion, chive and garlic crops, assessed by EWRS scores are presented in the Table 2.

From these results we draw the following conclusions:

- The herbicide Dual Gold controls very well the annual monocotyledonous weeds, *Setaria glauca* and *Digitaria sanguinalis*, as well as the annual dicotyledons *Stellaria media*, *Amaranthus retroflexus* and *Galinsoga parviflora*. It controls approx. 80% on *Chenopodium album* (EWRS score 2) and does not control the annual dicotyledons with large seeds: *Xanthium strumarium*, *Xanthium spinosum*, *Abutilon theophrasti* and *Ambrosia artemisiifolia*. It does not control perennial weeds and does not have any effect on their inhibition;
- The herbicide Pendisol 40 SC has a similar effect to Dual Gold yet, in addition, it controls *Chenopodium album* and *Ambrosia artemisiifolia*;
- The herbicide Galigan 240 EC, at a dose of 1 l/ha, controls very well the annual dicotyledons with small seeds but has a weaker effect on the annual monocotyledons *Setaria glauca* and *Digitaria sanguinalis*. At a dose of 2 l/ha it has a radical effect. However, it does not control large-seeded dicotyledons either;
- No crop showed phytotoxicity phenomena with the applied herbicides and doses.

Oxyfluorfen, metolachlor and pendimethalin are also recommended by other research conducted in our country and abroad for controlling weeds in sown onion crops, onion planted from bulbs or garlic (https://content.ces.ncsu.edu/weed-management-in-onions https://ipm.ucanr.edu/agriculture/onion-and-garlic/integrated-weed-management/#gsc.tab=0 https://www.canr.msu.edu/news/onion-weed-control-for-2019 https://www.dhanuka.com/blogs/post-emergence-herbicides-for-onion-crop https://agrointel.ro/261308/ebicid-ceapa-cand-cu-ce-stropim https://agro-bucuresti.ro/wp-content/uploads/2022/08/Agrotehnica_2001.pdf https://asas.ro/sectii/plante-camp/documente/premii/B1.TRATAT%20AGROTEHNICA%202020%20-pdf%20(1)-Copy.pdf https://acad.ro/sectii2002/proceedingsChemistry/doc2014-1/art07Gidea.pdf; Rădoi V. et. al., 1995).

b. **In the case of the herbicide Galigan 240 EC applied in post-emergence in different concentrations**

The results are presented in the Table 3.

From these results we draw the following conclusions:

- The concentration of 33 ml Galigan 240 EC in 5 liters of water can only be applied to sown onions when they grow large leaves, like onion planted from bulbs that are covered with wax. Although the herbicide leaflet does not specify not to apply to sown onions, in young stages, we observed obvious phytotoxicity phenomena, namely the total necrosis of the seedlings.
- The concentration of 33 ml Galigan 240 EC in 5 liters of water cannot be applied to garlic, in any growth phase, because garlic does not have wax on its leaves, as onion, it has less wax and for this reason it cannot withstand this concentration;
- The other concentrations, of 7 ml in 5 liters of water, 5 ml in 5 liters of water and 5 ml in 7 liters of water can be applied to sown onion and garlic, in cotyledons phase of the weeds;
- The resistant weeds to all concentrations were those with hairs on the leaves, with velvety or waxy leaves, such as purslane, velvetleaf and ragweed which do not allow the herbicide to enter the leaf;
- Perennial weeds as *Cirsium*, *Convolvulus*, *Sorghum* and *Cynodon* recover after approx. 2 weeks while the onion or garlic grows and can withstand another herbicide. Perennial monocots are controlled by specific herbicides: Agil, Fusilade, etc.
- If the treatment with Galigan is done on mature weeds, over 15 cm high, even at a concentration of 33 ml in 5 l of water, they recover after approx. 10 days.

Table 2. Efficacy and selectivity of herbicides applied to sown onion, planted onion from chive and garlic crops, expressed by EWRS scores

Weed Bayer Code	Biological category	V1	V2	V3	V4	V5	V6	V7	V8	V9
CIRAR	p.d.	9	9	9	9	9	9	9	9	9
CONAR	p.d.	9	9	9	9	9	9	9	9	9
CHEAL	a.d.	2	1	1	2	1	0	2	1	0
STEME	a.d.	1	1	1	1	1	0	1	1	0
AMARE	a.d.	1	1	1	1	1	0	1	1	0
POROL	a.d.	1	1	1	1	1	0	1	1	0
GASPA	a.d.	1	1	1	1	1	0	1	1	0
ABUTH	a.d.	9	9	9	9	9	9	9	9	9
AMBEL	a.d.	9	2	1	9	2	1	9	2	1
XANST	a.d.	9	9	9	9	9	9	9	9	9
XANSP	a.d.	9	9	9	9	9	9	9	9	9
SORHA	p.m.	9	9	9	9	9	9	9	9	9
CYNDA	p.m.	9	9	9	9	9	9	9	9	9
SETLU	a.m.	0	0	3	0	0	2	0	0	2
DIGSA	a.m.	0	0	3	0	0	2	0	0	2
Phytotoxicity		0	0	1	0	0	0	0	0	0

Legend: p.d. - perennial dicot; a.d. - annual dicot; p.m. - perennial monocot; a.m. - annual monocot. CIRAR - *Cirsium arvense*; CONAR - *Convolvulus arvensis*; CHEAL - *Chenopodium album*; STEME - *Stellaria media*; AMARE - *Amaranthus retroflexus*; POROL - *Portulaca oleracaea*; GASPA - *Galinsoga parviflora*; ABUTH - *Abutilon theophrasti*; AMBEL - *Ambrozia artemisiifolia*; XANST - *Xanthium strumarium*; XANSP - *Xanthium spinosum*; SORHA - *Sorghum halepnse*; CYNDA - *Cynodon dactylon*; SETLU - *Setaria glauca*; DIGSA - *Digitaria sanguinalis*.
EWRS scale for herbicide efficacy: (0-2 = control accepted as efficacy; 2-10, control not accepted as efficacy). For phytotoxicity on the crop: 0-2 low damage accepted; 2-10 strong damage, not accepted.

Table 3. Efficacy and selectivity of the herbicide Galigan 240 EC applied in 4 concentrations, on sown onion, onion planted from bulbs and garlic crops, expressed by EWRS scores

Weed Bayer Code	Biological category	V1		V2		V3		V4	
		3-4 leaves of the crop	8-10 leaves of the crop	3-4 leaves of the crop	8-10 leaves of the crop	3-4 leaves of the crop	8-10 leaves of the crop	3-4 leaves of the crop	8-10 leaves of the crop
CIRAR	p.d.	1	1	1	1	1	2	1	2
CONAR	p.d.	1	1	1	1	1	2	1	2
CHEAL	a.d.	1	1	1	1	1	2	1	2
STEME	a.d.	1	1	1	1	1	2	1	2
AMARE	a.d.	1	1	1	1	1	2	1	2
POROL	a.d.	3	5	4	6	4	6	6	7
GASPA	a.d.	1	1	1	1	1	2	1	2
ABUTH	a.d.	3	5	4	6	4	6	6	7
AMBEL	a.d.	1	1	1	1	1	2	1	2
XANST	a.d.	1	1	1	1	1	2	1	2
XANSP	a.d.	1	1	1	1	1	2	1	2
SORHA	p.m.	1	1	1	1	1	2	1	2
CYNDA	p.m.	3	5	4	6	4	6	6	7
SETLU	a.m.	1	1	1	1	1	2	1	2
DIGSA	a.m.	1	1	1	1	1	2	1	2
Phytotoxicity on sown onion		9	7	3	4	2	2	1	1
Phytotoxicity on onion planted from bulbs		3	2	1	1	1	1	1	1
Phytotoxicity on garlic		7	5	2	2	1	1	1	1

Legend: p.d. - perennial dicot; a.d. - annual dicot; p.m. - perennial monocot; a.m. - annual monocot. CIRAR - *Cirsium arvense*; CONAR - *Convolvulus arvensis*; CHEAL - *Chenopodium album*; STEME - *Stellaria media*; AMARE - *Amaranthus retroflexus*; POROL - *Portulaca oleracaea*; GASPA - *Galinsoga parviflora*; ABUTH - *Abutilon theophrasti*; AMBEL - *Ambrozia artemisiifolia*; XANST - Xanthium strumarium; XANSP - *Xanthium spinosum*; SORHA - *Sorghum halepnse*; CYNDA - *Cynodon dactylon*; SETLU - *Setaria glauca*; DIGSA - *Digitaria sanguinalis*.
EWRS scale for herbicide efficacy: (0-2 = control accepted as efficacy; 2-10, control not accepted as efficacy). For phytotoxicity on the crop: 0-2 low damage accepted; 2-10 strong damage, not accepted.

CONCLUSIONS

Onion and garlic cultivation requires weed control with herbicides to ensure economic efficiency. Three herbicide active ingredients were tested with pre-emergence application and four concentrations of oxyfluorfen in post-emergence. The herbicides tested after sowing or planting onion and garlic were: Dual Gold (metolachlor), Pendisol 40 SC (pendimethalin) and Galigan 240 EC (oxyfluorfen).

Regarding the application of the three herbicides to the three crops, it was observed that the herbicides Dual Gold and Pendisol 40 SC control very well annual monocotyledonous and annual dicotyledonous weeds with small seeds, such as *Setaria glauca*, *Stellaria media*, *Amaranthus retroflexus*, *Galinsoga parviflora* or *Portulaca oleracaea*. Weeds with large seeds, such as *Abutilon theophrasti*, *Xanthium strumarium* and *Ambrosia artemisiifolia* are not controlled by the Dual Gold and Galigan 240 EC treatments, but the herbicide Pendisol 40 SC controls the species *Ambrosia artemisiifolia* as well as *Chenopodium album*, compared to the Dual Gold herbicide. The herbicide Galigan 240 EC does not control annual monocotyledonous weeds. No herbicide applied to the soil controls perennial monocotyledonous or dicotyledonous weeds.

Regarding post-emergence treatments, it was observed that both sown onion and garlic do not tolerate the application of a concentration of 33 ml Galigan 240 EC in 5 liters of water (0.6%) or 2 liters of herbicide in 300 liters of water per hectare. This is not mentioned in the leaflet info of this herbicide.

The other oxyfluorfen concentration treatments must be applied when the weeds are young, small, otherwise they will regenerate their leaves in about 10 days.

The resistant weeds to all concentrations were those with hairs on the leaves, with velvety or waxy leaves, such as purslane, velvetleaf and ragweed which do not allow the herbicide to enter the leaf.

Perennial weeds as *Cirsium*, *Convolvulus*, *Sorghum* and *Cynodon* recover after approx. 2 weeks while the onion or garlic grows and can withstand another herbicide. Perennial monocots are controlled by specific herbicides: Agil, Fusilade, etc.

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RESEARCH ON THE INFLUENCE OF FERTILIZATION ON TOLERANCE TO ABIOTIC STRESS IN THE CASE OF SOME TOMATO CULTIVARS CULTIVATED IN THE SOUTHERN AREA OF ROMANIA

Simona Ana FORTAN, GiancarlaVELICEVICI, Dumitru Dorin CAMEN

University of Life Science "King Mihai I" from Timișoara, Faculty of Engineering and Applied Technologies, Department of Genetic Engineering, 119 Calea Aradului Street, Timișoara, Romania

Corresponding author email: fortan_ana@yahoo.com

Abstract

Tomatoes (Solanum lycopersicon) are some of the most cultivated and consumed vegetables worldwide, having significant economic and nutritional importance. Tomatoes are some of the most important horticultural crops in Romania, but production is often affected by abiotic stress factors, characteristics this region. This research aimed to analyse how fertilization influences tolerance to abiotic stress (such as drought, salinity or extreme temperatures) in the case of some tomato cultivars grown in southern Romania, namely Pontica (Dacia), Florina 44 and Buzau 1600. The study focused on the evaluation of different types of fertilizers and their impact on the response of plants to stressful conditions. The obtained results of this research refer to the identification of optimal fertilization practices that can improve stress tolerance, and the determination of some tomato cultivars that show a superior resistance to adverse environmental conditions, offering valuable perspectives for the development of sustainable agricultural practices in the southwest region of the countries.

Key words*: abiotic stress, tomato, tolerant, foliar fertilization, southern Romania.*

INTRODUCTION

Tomatoes represent some of the most valuable vegetables from a food point of view. They are a good source of phytochemicals and nutrients such as lycopene, potassium, iron, folate and vitamin C (Bădulescu et al., 2020); Demidchik, (2018). In addition to lycopene and vitamin C, tomatoes also provide other antioxidants such as beta-carotene and phenolic compounds such as flavonoids, hydroxycinnamic acid, chlorogenic acid, homovanillic acid and ferulic acid (Cuc et al., 2015). Abiotic stress factors, such as drought, salinity, and extreme temperatures, significantly affect the growth and yield of tomato plants. Fertilization, particularly foliar and soil treatments, plays a crucial role in enhancing the tolerance of tomatoes to these stresses. This article examines the impact of various fertilization strategies on tomato cultivars, with a focus on the mechanisms that improve resistance to abiotic stresses. The results indicate that optimized fertilization, including the use of bio stimulants and essential nutrients, can mitigate the detrimental effects of environmental stress, resulting in improved plant growth and yield (Khan, 2015). The qualities they possess have caused them to be consumed in the most varied regions of the globe, even where they are not cultivated. The increase in tomato consumption is due to the fact that these vegetables have a very pleasant taste and an incredibly varied range of uses (fresh, in the form of tomato salad or mixed with other vegetables, soups, broth, pots, sauces, stuffed tomatoes, etc.). In the context of climate change and the intensification of extreme phenomena, abiotic stress has become a major problem for agriculture. Plant stress tolerance can be influenced by fertilization techniques, which can help crops better cope with adverse conditions. Drought among various abiotic stresses, is one of the basic factors for restricting crops production (Vallivodan, B., & Nguyen, H.T. 2006; Demidchik, V. 2018). It is predicted that one third of the world population will be threatened by water shortage in the year 2025 (Mahlagha et al., 2012). Various photosynthesis mechanisms and metabolic activities require water (Oo et al., 2020). Additionally, to maintain their growing performance, maximum amount of water is required by the plants (Tátrai et al., 2016).

Drought stress negatively affects the physiological, genetic, biochemical, and morphological characteristics of plants (Torres-Ruiz et al., 2015) Drought stress restricts plant growth by decreasing photosynthetic rate. Regarding photosynthesis in leaves, chlorophyll fluorescence reflects the intrinsic characteristics of this. There are some studies have been carried out on the photosynthesis of tomato under drought stress but are not comprehensive (Guoting et al., 2020; Brix, 2010; Jangid K.K., & Dwivedi V., 2016). Chlorophyll fluorescence technique is useful as a non-invasive tool in eco-physiological studies and has extensively been used in assessing plant responses to environmental stress (Parry et al., 2006). Plants growing under natural conditions are exposed to a variety of abiotic stresses, which adversely affect their development and performance due to the inhibition of a number of physiological and metabolic processes (Easwar R.D. & Chaitanya K.V., 2016). The light source plays an important role in the growth and development of tomatoes, providing energy for tomatoes on the one hand, and regulating tomato plant morphology on the other hand (Li Y et al., 2021). In plants, solar energy is converted into chemical energy by the complex process of photosynthesis. Crop production is strongly dependent on the photosynthetic rates. Generally, plants try to maintain photosynthetic efficiency under changing light intensities by balancing conversion of radiation energy and protecting any damage to photosynthetic apparatus by photoinhibition and repairing damage (Wimalasekera R., 2019). This research focuses on; identification of tomato cultivars that show a higher tolerance to abiotic stress, evaluation of the types of fertilizers (organic, mineral, combined) and their application in different phases of plant growth, observing the effect on studied parameters.

MATERIALS AND METHODS

The study was done in the year, 2024, in the south-west of Oltenia, more exactly in Varvoru de Jos commune, Dolj County The biological material used in this study was represented by three cultivars of tomato: Pontica (Dacica), Buzău 1600 and Florina 44. Pontica (Dacia) is a Romanian tomato variety favored by many growers. This variety offers a summer-autumn crop with determinate growth. The plants develop a single row of fruit, but the productivity is very high, 90-100 tons per hectare. You can easily establish a crop either by direct seeding or transplanting, without the need for additional care like in the case of indeterminate tomatoes. Pontica is also characterized by its drought tolerance and adaptability to different soil types. It is a semi-late and highly productive crop, with yields reaching 90-100 t/ha. The vegetative period lasts 110-120 days. The stem is vigorous and reaches a height of 60-70 cm. The fruits are round and can weigh around 90-150 grams. Buzău 1600 tomatoes are intended for growers who want indeterminate tomatoes, to be cultivated on stakes, both in open fields and in protected environments such as greenhouses or tunnels. The tomatoes are large, meaty, and very flavourful. Their average weight is around 200-250 grams. The fruits are resistant to cracking.

One seed packet is enough for approximately 200 plants. Sowing: Buzău 1600 tomatoes should be sown in seedbeds, pots, or seedling trays between February and March. Sow in February if you plan to plant them in greenhouses, or in March if planting them in open fields (garden).

Germination conditions: The plants need access to daylight and a temperature of 20-22°C to germinate properly. Transplanting: Seedlings can be transplanted into the garden or greenhouse after about 40 days. Plant them 90 cm between rows and 25-50 cm between plants in a row.

Direct sowing in the field: Direct sowing involves planting the seeds directly into the garden when the germination conditions are met usually around May. [20:14, 21.04.2025] Chat Gpt: Florina 44 Tomato. The Florina 44 tomato variety was developed at the Vegetable Research and Development Station (SCDL) Buzău as a result of research in tomato breeding carried out between 1996 and 2016. The newly developed variety was patented and added to the official list of cultivated plants in Romania starting in 2017.

It is a determinate growth variety (Sp), intended for open field cultivation. It was

obtained from line 44 through intensive breeding efforts. The plant grows as a bushy type, consisting of 4-6 main stems with an average height of 60-65 cm.

Plant vigor is medium, with 60-80 leaves featuring medium-sized leaflets. The immature fruit is green with a slight shoulder, and at physiological maturity, it turns bright red. Each inflorescence bears 4-6 large, round fruits, with an average fruit weight of 180-220 grams. The fruits are firm, resistant to cracking and sunburn, and have good post-harvest shelf life (over 10 days). A cross-sectional cut reveals a pericarp thickness of 7-8 mm and 4 seed cavities (locules) [20:14, 21.04.2025] Chat Gpt: - Each fruit contains a small number of well-developed seeds, around 60-80, which are covered with fine golden hairs. The fruit has an attractive commercial appearance and a pleasant, balanced taste. Fruits can also be harvested with the peduncle attached, thanks to their short pedicel, which improves post-harvest longevity. Economic Efficiency: Production potential: 50-60 tons/ha High quality and commercial value.

Florina 44 can be cultivated in all regions of the country that are favourable for open-field tomato cultivation. Its exceptional flavour and aroma make it ideal for fresh consumption, while its dry matter content of over 6.2% also makes it suitable for processing and industrial use. The Florina 44 and Buzău 1600 tomato varieties were developed at the Vegetable Research and Development Station (SCDL) Buzău. Pontica (Dacia) is a Romanian semi-early tomato variety created by ICDLF Vidra in 1988 and re-approved in 2009.

The measurement of growth was carried out through linear measurements at different time intervals, every 7 days, expressed in centi-metres to determine photosynthesis, what is actually measured is the Photosynthetic Efficiency (based on cyclic photophosphorylation), starting from the premise that the highest efficiency (the highest potential for photon acceptance) corresponds to dark conditions - when the electron transport chain should be free of electrons. This Photosynthetic Efficiency can be expressed as a percentage (the reasoning is presented in the attached material). Photosynthesis measuring devices, based on chlorophyll fluorescence, have developed since the beginning of the 1990´s. EARS, in Delft, the Netherlands, was the first company that developed a handheld instrument. This plant photosynthesis meter (PPM) has since then been improved and developed further. So far the portable instrument was still of substantial size. But in 2011 the miniPPM was launched. This instrument is based on a novel measuring % method, has excellent measuring properties, but is not much larger than a mobile phone. Moreover the instrument is very affordable. As a result it is expected to reach a larger target group, in particular farmers and greenhouse growers. But the miniPPM is also very suitable for schools and in applied research. For the experiments the plants were grown in field condition. The experimental variants: Factors that were studied during the experiment:

- for intensity of plant growth:

Factor A [phenophase] $p<0.001$:a1-18.05.2024, a2-25.05.2024, a3-01.06.2024, a4-08.06.2024, a5-16.06.2024, a6-20.06.2024, a7-07.07.2024. Factor B [genotype] $p>0.05$:Pontica (Dacica), Buzău 1600 and Florina 44. The null hypothesis H0 for factor A [phenophase] is rejected, and the null H0 hypothesis for factor B[genotype] is accepted.

- for intensity of photosynthesis:

Factor A [phenophase];

Factor B [genotyp]:

Factor C[varianta]: **V0** - Atonik 10 ml/10 l; **V1** - Atonik 10 ml/10 l + Albit 2 ml/10 l + Albit 2 ml/10 l;

V2 - Atonik 10 ml/10 l + Albit 2 ml/10 l + Albit 2 ml /10 l + Poliamin/50/10 l + Poliamin /100/10 l + Poliamin /100/10 l.

Albit is a substance with a protective and stimulating role, positively influencing all plant life functions, contributing to strong immunity and a balanced metabolism. It is used in over 60 types of crops. It is based on natural combat mechanisms and contains substances synthe-sized by beneficial soil bacteria. Its compo-sition also includes a set of substances that enhance and amplify the effect of PHB: MgO, SO4, K2O, P2O5, and N. Albit is used together with chemical pesticides to reduce their stressful effect and to increase their efficiency

Apply 10 ml of the product in 10 liters of water. Albit was applied when the plants had 3-4 leaves Atonik is a biochemical stimulator for:

growth, fruiting, rooting, and the stimulation of seed germination in horticultural plants.
Atonik stimulates rooting, seed and pollen germination, pollen tube growth, shoot proliferation and development, flower fertility, and fruit formation. In tomato cultivation, in order to achieve increased yield, the product is applied at a dose of 0.5 L/ha. Haifa Poly-Amin is a natural bio-stimulant, specially designed for foliar application. It contains amino acids and low molecular weight peptides, which act synergistically to catalyse growth processes and support the plant's metabolism. It is applied two to three times, every two weeks. Apply 100 ml of the product in 10 litters of water.
The fertilization was applied in different phenophases of plant growth. Variant 0 - Fertilization at this stage was done with Atonik when the plants had 10 leaves, on May 20, 2024.
Variant 1 - The first fertilizer applied was Atonik, on May 20, 2024, at the 10-leaf stage. The second fertilizer was Albit, applied on the following dates: June 5, 2024, June 12, 2024, and July 12, 2024.
Variant 2 - The first fertilizer applied was Atonik, on May 20, 2024, at the 10-leaf stage. The second fertilizer was Albit, applied on June 5, 2024, June 12, 2024, and July 12, 2024. The third fertilizer was Polyamin, applied three times on the following dates: June 11, 2024, June 26, 2024, and July 7, 2024. The parameters determined applied research methods were: determining the intensity of plant height, determining the intensity of photosynthesis. Experimental data have been processed by statistical methods: using analysis of variance (ANOVA) for 95% confidence level and DUNCAN test to determine the significance of differences.

RESULTS AND DISCUSSIONS

Given the results in Table 1, we can observe that the phenophase have a significant influence upon or on growth intensity evaluated by the plant height evalution.
As there are no significant differences between cultivars in terms of plant height, we looked for an adequate equation between phenophase and plant height - the average of cultivars. The equation sought was an exponential equation, i.e. the trend of development of the plant size is exponential depending on the phenophase. The exponential equation best approximates the evolution of plant growth as a function of phenophase. We can present 3 equations: linear, parabolic, exponential.

Table 1. Variance analysis of growth intensity

Source of variation	SSP [SP]	Degrees of freedom	Weighted sum of squares [s^2]	F-test vs. s2error		
				the value	p	the meaning
phenophase	15333.90	6	2555.65	24.8389	0.000004	***
genotype	190.17	2	95.08	0.9241	0.423364	ns
Error	1234.67	12	102.89			
Total	16758.74					

ns $p>0.05$; *$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.001$

By comparing them, we can choose the one that best approximates the phenomenon studied [it all boils down to the analysis of the variance analysis table for examining the respective correlation - test F].
From the Table 2 we can see the influence of phenophase upon plants height. From this point of wiew, the best influence was registered to a7 phenophase (90).

Table 2. The influence of phenophase upon plants height

Phenophase	(Mean(Cm)	Std.Err.	-95.00%	+95.00%
a1-18.05.2024	6.33	0.33333	4.89912	7.7676
a2-25.05.2024	8.67	0.16667	7.94956	9.3838
a3-01.06.2024	11.33	0.33333	9.89912	12.7676
a4-08.06.2024	25.00			
a5-16.06.2024	33.67	1.85592	25.68128	41.6521
a6-20.06.2024	40.67	0.66667	37.79823	43.5351
a7-07.07.2024	90.00	15.27525	24.27589	155.7241

The cultivars studied showed mean values of this character between 28.07 cm in Florina 44 and 35 cm in Buzau genotype.

Table 3.The influence of phenophase uponcultivars studied

Cultivars	Mean (cm)	Std. Err.	-95.00%	+95.00%
b1 - Buzau	35.00	14.95071	-1.58308	71.58308
b2 - Pontica	29.36	9.81539	5.33975	53.37453
b3 - Florina 44	28.07	8.63853	6.93371	49.20914

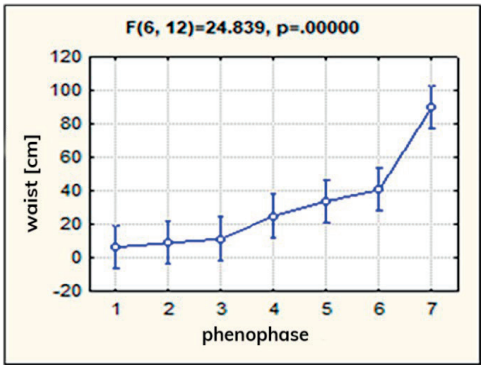


Figure 1. Influence of phenophaseupon plant height

The statistical results regarding the influence of genotype on plant height are presented in Figure 2.

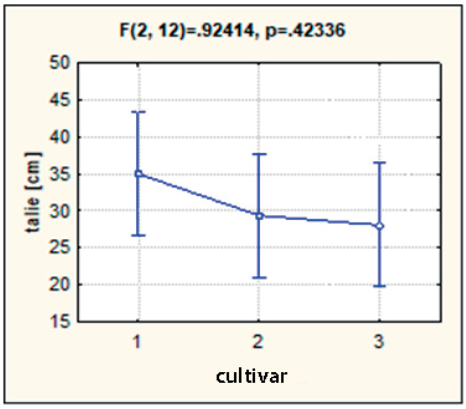


Figure 2. The influence of genotype upon plant height
ns p>0.05; *p≤0.05;**p≤0.01;***p≤0.001

Table 4.Variance analysis for intensity of photosynthesis

	SSP			Test F		
Source of variation	**[SP]**	**Degrees of freedom**	**[s^2]**	**value**	**p**	**Signi ficance**
				5.21	0.005524	
[phenophase]	136.6	3	45.5	0.46	0.633607	**
[cultivars]	8.1	2	4.1	1.28	0.294133	ns
[variants]	22.4	2	11.2			ns
Error	244.9	28	8.7			
Total	411.9					

From the Table 4 we can observe that the phenophase had a statistically assured action.
The cultivars and fertilization treatments have an action without statistical assured.
The null hypothesis H0 for phenophase is rejected, and the null H0 hypothesis for factor genotype and fertilization variants were accepted.

Table 5a. The influence of phenophase upon intensity of photosynthesis

Phenophase	Mean %	Std. Err.	-95.00%	+95.00%	N
a1 – 20.06.2024	72.80	0.360555	71.96856	73.63144	9
a2 – 26.06.2024	71.24	0.955556	69.04093	73.44796	9
a3 – 01.07.2024	76.58	1.368878	73.42114	79.73442	9
a4 – 29.07.2024	73.96	0.952489	71.76652	76.15941	9
Mean	73.65				

The average intensity of photosynthesis was between 71.24%, in second period and 73.96%, in fourth period (Table 5a).
Low light intensity influences photosynthesis, which is central to plant productivity, and can therefore severely restrict plant growthand even death (Zhu et al., 2014; Wang et al., 2021).

Table 5b. The influence of cultivars upon intensity of photosynthesis

Cultivars	Mean %	Std. Err.	-95.00%	+95.0%	N
b1 - Buzau	73.56	0.687982	72.04410	75.07257	12
b2 - Pontica/Daci a	73.11	1.047383	70.80861	75.41916	12
b3 - Florina 44	74.27	1.220262	71.58089	76.95245	12

The best influence upon yield of photosynthesis had the Florina 44 (74.27%) (Table 5b). The results regarding the influence of fertilization treatmentson the intensity of photosynthesis during the 2024 year are presented in Table 6.

Table 6. The influence of fertilization treatments upon intensity of photosynthesis

Variant	Mean (%)	Std. Err.	-95.00%	+95.00%	N
c1 - V0	72.58	0.774774	70.87251	74.28304	12
c2 - V1	74.46	0.744851	72.81615	76.09496	12
c2 - V2	73.91	1.340265	70.95565	76.85546	12

The best fertilization treatment was V1. The application of this treatment option stimulated photosynthesis and intensification of plant metabolism, reducing oxidative stress. Foliar fertilization helps to quickly correct deficiencies and improves photosynthetic efficiency. The application of this mixture led to more vigorous plants with improved production and increased resistance to stressors.

CONCLUSIONS

The height of tomato plants is influenced not only by genotype, but also by the phenophase of growth. Cultivars control tomato size, plant architecture, and finally crop yield. The Buzau genotype recorded the largest plant hight.
Each phenophase has different requirements and influences plant growth. Proper management of water, nutrients, and temperature ensures healthy development and high production.
The best fertilization treatment was V1. Application stimulated photosynthesis and enhanced plant metabolism, reducing oxidative stress.

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STUDY ON THE IDENTIFICATION OF *AMARANTHUS* VARIETIES FOR FRESH CONSUMPTION

Luminița GĂITĂNARU[1], Costel VÎNĂTORU[2], Elena DOBRIN[1], Elena Maria DRĂGHICI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Plant Genetic Resources Bank for Vegetables, Floricultural,
Aromatic and Medicinal Plants Buzău, Romania

Corresponding author emails: draghiciem@yahoo.com; elena.dobrin@gmail.com

Abstract

Amaranthus spp. has demonstrated a special vitality, growing in many types of environments, the genus having pioneer species that have found unoccupied niches in nature, such as colonizing affected lands. It produces many seeds that germinate quickly, staggered and the productivity is exceptional, allowing several harvests per year. The leaves are edible and have a taste similar to loboda (Atriplex hortensis), being more succulent and making culinary preparations denser. It has a tendency to assimilate nitrogen in the form of nitrates and cultivated on soils on which excess nitrogen fertilizers have been applied, it becomes toxic to humans and animals, such as cattle and pigs, causing indigestion and bloating. The study aims to analyze and identify Amaranthus varieties that are recommended for consumption.

Key words*: amaranth, cultivars, identification, nutrients.*

INTRODUCTION

Amaranthus, known as "știr" in Romanian, is a plant with a long history of use in nutrition due to its high nutritional value and adaptability to various environmental conditions. In recent years, research has focused on identifying *Amaranthus* varieties suitable for fresh consumption, considering their health benefits and potential to diversify the human diet (Popescu, 2021).

These crops are highly nutritious and environmentally resistant. They can be adapted to different environmental conditions, being cultivated in poor soils and high altitudes (Valcárcel-Yamani & Caetano, 2012).

Studies have shown that different varieties of *Amaranthus* exhibit significant variations in terms of phytochemical composition and antioxidant activity. For example, research has indicated that "amaranth seeds represent a potentially rich source of polyphenols and gluten-free protein compounds, with cultivars such as Hopi Red Dye being the richest in such compounds" (Ionescu & Radu, 2020).

Additionally, amaranth is recognized for its high content of antioxidants, such as gallic acid, p-hydroxybenzoic acid, and vanillic acid, which can help protect against diseases (Dumitru et al., 2019).

These antioxidant properties contribute to reducing inflammation and preventing chronic conditions, making amaranth a valuable component of modern diets (Mihai, 2022).

Amaranth foliage is used as a vegetable and as animal feed, especially in the tropics and subtropics. It is an excellent source of bioavailable iron (Rangarajan & Kelly, 1994).

Given these aspects, identifying and cultivating *Amaranthus* varieties suitable for fresh consumption represents an opportunity to improve dietary diversity and promote healthy nutrition. It is essential to continue research to evaluate the potential of these varieties in different agroecological conditions and to fully understand their benefits for human health (Georgescu & Marin, 2023).

MATERIALS AND METHODS

Study area

The study was conducted within the Bucov Botanical Garden, located in Prahova County, Romania.

This location was chosen due to the favorable pedoclimatic conditions for cultivating species

of the genus *Amaranthus*. The soil in this area is cambic chernozem, with a medium texture, moderate humus content, and a neutral pH (6.5-7.0).

The climate is temperate continental, with an average annual temperature of approximately 10°C and an average annual precipitation ranging between 650 and 700 mm.

Biological Material

In this study, five lines of *Amaranthus* were used, selected for both their food value and ornamental value:

L1 - *Amaranthus cruentus* (food variety, known as "red știr");

L2 - *Amaranthus cruentus* (food variety, intended for popcorn production)

L3 - *Amaranthus caudatus red* (food and ornamental variety);

L4 - *Amaranthus retroflexus* (food and energy variety);

L5 - *Amaranthus caudatus* (ornamental variety).

The seeds of each line were selected from certified batches and tested for viability before sowing.

The experiment was conducted in open-field conditions, following a completely randomized experimental design with three replications for each line.

Sowing was carried out at the end of April, using a row spacing of 50 cm and 15 cm between plants within a row.

During the growing season, the following technological measures were applied:

Irrigation - drip irrigation was used to ensure consistent soil moisture.

Fertilization - organic compost-based fertilizer was applied at a rate of 10 t/ha.

Weed control - manual weeding was performed to maintain the ecological nature of the crop.

Parameters analyzed

For each *Amaranthus* line, the following agronomic and nutritional parameters were monitored: plant height, number of leaves, and leaf area.

Statistical analysis

The experimental data were processed using analysis of variance (ANOVA), and significant differences between lines were tested using Duncan's test ($p < 0.05$). The software used for statistical processing was SPSS.

RESULTS AND DISCUSSIONS

The analysis of morphological and physiological parameters of different *Amaranthus* lines is essential for identifying the most suitable varieties intended for fresh consumption. In this study, characteristics such as plant height, branching, leaf size, and inflorescence length were evaluated, considering their impact on yield and production quality.

The results obtained highlight significant differences between the analyzed lines, each having specific traits that can influence both agricultural production and food use.

Below, we will present and interpret the data, emphasizing the strengths and limitations of each variety.

Plant Height

L4 and L3 were the tallest lines, with average heights of 111 cm.

L1 had the smallest height (78 cm), which may indicate slower growth or a different adaptation to environmental conditions (Table 1).

Stem Height

L3 and L5 had taller stems, 11.6 cm and 11.8 cm, respectively, which may indicate a more robust plant structure.

L1, L2 and L4 had stems shorter than 3 cm, 2 cm and 2.4 cm, respectively, which may indicate a difference in plant architecture

Table 1. Plant and stem height

Lines	Plant height (cm)	Stem height (cm)
L1	78 ± 15^{a}	3 ± 0^{a}
L2	107 ± 5^{b}	2 ± 0^{a}
L3	111 ± 16^{b}	11.6 ± 2.3^{b}
L4	111 ± 27^{b}	2.4 ± 0.5^{a}
L5	97 ± 18^{a}	11.8 ± 1.4^{b}

Number of Primary and Secondary Shoots and bush diameter can be observed in Table 2.

L2 had the highest number of primary shoots (13.4), suggesting a high branching capacity.

L4 dominates in the number of secondary shoots (21.8), which may indicate good regeneration potential and higher plant density (Table 2).

L4 had the largest bush diameter (103 cm), which requires planting to be carried out at a greater distance between plants.
L1 and L2 had smaller dimensions (59 cm and 74 cm, respectively), which can influence the plant biomass but also the planting distances (Table 2).

Table 2. Number of shoots and bush diameter

Lines	Main shoots (no.)	Secondary shoots (no.)	Bush diameter (cm)
L1	3 ± 0^{ab}	8.8 ± 0.8^{a}	59 ± 10.6^{a}
L2	13.4 ± 13.4^{d}	6.6 ± 1.51^{a}	74 ± 11^{ab}
L3	3.6 ± 0.5^{b}	14.6 ± 2.9^{b}	99 ± 25^{a}
L4	10.8 ± 0.8^{c}	21.8 ± 1.7^{c}	103 ± 27^{a}
L5	2.6 ± 0.5^{a}	8.6 ± 1.3^{a}	88 ± 24^{ab}

Number of Leaves
L4 had the highest number of leaves (202 leaves), suggesting a high photosynthetic potential.
L5 had the lowest number of leaves (90.4 leaves), which may affect total biomass production.
Leaf Length
L3 and L4 had longer leaves (5.8 cm), which could make them more attractive for food consumption. The other lines have smaller leaves, L1 with 4.3 and L2 4.6 cm), which may influence texture and harvesting yield (Table 3).

Table 3. Number of leaves on plants

Lines	Leaves no.	Leaf length (cm)	Distance between leaves/plant (cm)
L1	186 ± 20^{c}	4.3 ± 0.6^{b}	8.4 ± 1.1^{b}
L2	160 ± 55^{bc}	4.6 ± 0.4^{b}	0.5 ± 0.5^{a}
L3	120 ± 12^{ab}	5.8 ± 0.9^{a}	1.4 ± 1.4^{b}
L4	202 ± 59^{c}	5.8 ± 1^{a}	0.8 ± 0.8^{a}
L5	90.4 ± 8.2^{a}	4.6 ± 0.4^{b}	2.5 ± 2.5^{c}

Distance Between Leaves on the Plant
L1 had the greatest distance between leaves (8.4 cm), indicating a more open plant structure.
L4 and L2 had the smallest distances between leaves (0.8 cm, respectively 0.5 cm), which may favor denser foliage.
Inflorescence Length
L4 had the longest inflorescences (46 cm), which could be advantageous for reproduction and seed production.
L2 and L3 had shorter inflorescences (30 cm respectively 7 cm) (Table 4).

Number of Spikes per Plant
L4 had the highest number of spikes (18.8), indicating a high seed yield potential. This line appears to be the most vigorous line, with the greatest height, the most leaves, and the highest number of spikes, suggesting high potential for biomass and seed production.

Table 4. The inflorescence length and number of spikes on plant

Lines	Inflorescence length (cm)	Spikes no./plant
L1	32 ± 6.7^{a}	7.4 ± 1.1^{a}
L2	30 ± 2.4^{a}	14.8 ± 2^{c}
L3	7.7 ± 7.7^{b}	13.4 ± 3.3^{bc}
L4	46 ± 5.1^{b}	18.8 ± 3.2^{d}
L5	14.4 ± 14.4^{b}	10.6 ± 1.6^{ab}

The images in the study illustrate the morphological characteristics of the different *Amaranthus* lines, highlighting significant variations in plant height, leaf size and number, inflorescence length and stem structure.
Figures 1-5 present *Amaranthus* plants differences in branching, leaf density and production potential.



Figure 1. L1 - *Amaranthus cruentus* (food variety, known as "red ştir")



Figure 2. L2 - *Amaranthus cruentus*
(food variety, intended for popcorn production)



Figure 3. L3 - *Amaranthus caudatus red*
(food and ornamental variety)



Figure 4. L4 - *Amaranthus retroflexus*
(food and energy variety)



Figure 5. L5 - *Amaranthus caudatus*
(ornamental variety)

CONCLUSIONS

It can be appreciated that the study on the identification of *Amaranthus* varieties for fresh consumption is very relevant for both the horticultural and food sectors. *Amaranthus* is a plant with multiple uses, being appreciated both for its nutrient-rich leaves and for its seeds. Therefore, the identification of the most suitable varieties for fresh consumption can contribute to increasing the diversity of products on the market but also to diversifying sustainable food sources.

The study on different *Amaranthus* lines intended for fresh consumption highlighted significant variations in the morphological and physiological characteristics of the plants. Based on the results obtained, the following conclusions can be drawn:

- *L4* stood out for its greatest height, high number of leaves, and spikes, making it a promising variety for biomass and seed production.
- *L2* demonstrated a high branching capacity, with the highest number of primary shoots, which may positively influence plant regeneration and crop yield.
- *L3 and L4* had longer leaves, which could be an advantage for fresh consumption.
- *L1 and L5* had the lowest number of spikes and leaves, which may limit their productivity compared to the other lines.
- Lines with dense foliage and vigorous growth (*L4, L2*) are the most suitable for intensive agricultural exploitation.
- *L5*, although less productive, may have ornamental applications or be used in mixed crops due to its distinctive appearance.

The results of this study confirm the need for rigorous selection of *Amaranthus* lines to optimize both agronomic yield and nutritional value.

In the future, additional studies on the phytochemical composition and health impact are necessary to confirm the benefits of consuming these varieties.

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EXPLORING THE SYNERGISTIC EFFECTS OF CALCIUM NITRATE AND BIOSTIMULANTS ON SEED YIELD IN *CAPSICUM ANNUUM* L. VAR. *GROSSUM*

Mariana Cristiana GHEORGHE[1], Delia Cristina CONSTANTIN[2], Mihaela PARASCHIV[2], Maria DINU[3]

[1]University of Craiova, Faculty of Horticulture, Doctoral School of Plant and Animal Resources Engineering, 13 A.I. Cuza Street, 200585, Craiova, Dolj County, Romania
[2]Research & Development Institute for Vegetable and Flower Growing, 22 Calea București, Vidra 077185, Ilfov County, Romania
[3]University of Craiova, Faculty of Horticulture, 13 A.I. Cuza Street, 200585, Craiova, Dolj County, Romania

Corresponding author email: mihaela1mail@yahoo.ca

Abstract

This work aims to study the potential for increasing seed yield in round pepper (Capsicum annuum L. var. grossum) by applying calcium nitrate in combination with various biostimulants. The biological material used in the experiment was the semi-early cultivar "Asteroid 204", obtained from ICDLF Vidra. Treatments included calcium nitrate alone and in combination with different biostimulants, specifically two seaweed extracts - one containing Ascophyllum nodosum and the other containing Ecklonia maxima - as well as two inocula based on mycorrhizal fungi, one enriched with Trichoderma spp. and the other with Bacillus spp. The results indicate that the combination of calcium nitrate and biostimulants - either seaweed-based or mycorrhizal fungi-based - can lead to an increase in seed yield and improvements in seed quality.

Key words*: biostimulants, mycorrhizal fungi, seed quality.*

INTRODUCTION

The vegetable sector faces difficulties in increasing vegetable yields, and reducing harmful effects on the environment and human health is a significant challenge. In conventional agriculture, modern practices have become increasingly limited due to environmental damage and the depletion of natural resources. Nowadays, agriculture relies heavily on the use of less harmful products, aiming to minimize chemical inputs (Almășan and Datcu, 2022).

Biostimulants are increasingly used to improve crop yield and quality. These products have diverse chemical compositions, often containing a mixture of organic and inorganic compounds, including essential macro- and micronutrients. The main categories of biostimulants include humic and fulvic acids, protein hydrolysates, seaweed extracts, chitosan, and inorganic compounds. They significantly contribute to enhancing plant growth, resistance, and productivity by stimulating natural physiological processes (Du Jardin, 2015; Singh et al., 2025; Apostol et al., 2022).

Seaweed extracts, rich in auxins and cytokinins, promote plant growth, flowering, and shelf life (Yao et al., 2020). Studies have shown that these extracts can significantly increase the height and number of branches in peppers (Sridhar & Rengasamy, 2012; Ozbay and Demirkiran, 2019; Vijayakumar et al., 2019; Ashour et al., 2021; Azzam et al., 2022).

Brown algae biostimulants, such as *Ecklonia maxima* and *Ascophyllum nodosum*, contribute to increased eggplant (Constantin et al., 2023a) and seed production. *Ecklonia maxima* treatments can lead to higher seed production in eggplant, although the response may vary among cultivars. *Ecklonia maxima*, *Ascophyllum nodosum*, and *Laminaria digitata* can also improve the seed vigor index in eggplant (Dascălu et al., 2025). Seaweed treatments can enhance the seed vigor index

when used as seed priming treatments (Constantin et al., 2023b). Additionally, *Ascophyllum nodosum* and calcium nitrate can increase fruit number per plant, seed production in peppers, and improve the seed vigor index in peppers (Constantin et al., 2025). The use of foliar treatments with *Ascophyllum nodosum* and calcium nitrate increased pepper and seed production when applied more frequently (at 7 or 10-day intervals), with treatments applied every 7 days also positively affecting the seed vigor index (Buzatu et al., 2024).

Biostimulant treatments in peppers have shown a significant increase in the number of seeds per fruit, as well as a significant increase in seed weight. This is mainly due to improved nutrient uptake and accessibility to the plants (Deori et al., 2023).

Calcium ions are essential nutrients for healthy plant growth and development, contributing to fruit quality and yield (Ragab et al., 2021). They play a crucial role in the formation of the plant cell wall and function as signaling molecules, influencing various physiological and pathological processes (Malinovsky et al., 2014). The application of calcium sources, such as calcium sulfate and calcium nitrate, can improve soil properties and increase nutrient availability, proving effective in saline soils (Khaled et al., 2019). Calcium stimulates root development, accelerates crop maturity, supports flowering and seed production, confers cold resistance, and promotes cell division (Sandhya, 2014). A low calcium concentration in plant tissues can cause physiological disorders such as blossom end rot in peppers, tomatoes, and eggplants, especially during periods of maximum fruit growth. The absorption of calcium ions is genetically influenced and depends on soil conditions, as well as biological and climatic factors (Shakoor & Bhat, 2014).

The aim of this work was to study the possibility of improving seed yield in round pepper by using calcium nitrate combined with biostimulants of different origins.

MATERIALS AND METHODS

In this study, the germination process of bell pepper seeds of the Asteroid 204 cultivar, produced at the Research and Development Institute for Vegetable and Flower Growing Vidra, was monitored under conditions of fertilization with various biostimulant products. Specifically, calcium nitrate was applied alone and in combination with four different biostimulants.

The chemical composition of these products and their respective concentrations used in the treatments are presented in Table 1. The treatments were applied every 10 days, beginning with the formation of the first fruits.

Table 1. Treatment applications and their compositions

Product	Chemical composition	Concentration used
Calcinit	Total nitrogen - 15.5; calcium oxide CaO 26.5%	5 g/l
E-Dalgin	Pure extract of *Ascophyllum nodosum*	2 ml/l
Kelpak	Pure extract of *Ecklonia maxima*	2 ml/l
Triptolemus HV	Mycorrhiza content: 0.1%; *Rhizosphere* bacteria content: 1 x 10^5 CFU/g; *Trichoderma* spp. content: 1.2 x 10^8 CFU/g.	3 ml/l
Albit	Poly-beta-hydroxybutyric acid; natural biopolymer synthesized from soil bacteria: *Bacillus magaterium* and *Pseudomonas aureofaciens*, min. 0.62%; Nitrogen min. 7.5%; Phosphorus min. 6%; Potassium min. 4.5%; Magnesium min. 0.6%; Sulfur min. 2.7%	0.1 ml/l

The experimental variants were: V1 - untreated; V2 - treated with calcium nitrate; V3 - treated with calcium nitrate and E-Dalgin; V4 - treated with calcium nitrate and Kelpak; V5 - treated with calcium nitrate and Triptolemus HV; V6 - treated with calcium nitrate and Albit.

The experimental variants were arranged in the field according to the experimental technique, using randomized block design, with three repetitions. The surface area of each experimental plot was 13 m². Planting was performed on May 16 using 60-day-old seedlings.

Fruits were harvested at physiological maturity, indicated by their red color, and the number of fruits per plant reaching physiological maturity was recorded. Three harvests were conducted (September 1, 15, and 30). Seed extraction was performed after cutting the fruits, removing the

receptacles, and detaching the seeds. Drying was carried out by exposing the seeds to the sun for 4 hours, followed by drying in a dry, ventilated room.

After conditioning the seeds in the selector, seed yield, seeds per fruit, and 1000-seed weight were calculated for each sample. The study was conducted under controlled laboratory conditions. Seeds were germinated in an incubator at alternating temperatures of 20°C for 16 hours and 30°C for 8 hours. For each treatment, six replicates of 100 seeds each were germinated (STAS **SR 1634/1999. Seeds for sowing. Germination test).

Seeds were evenly distributed on filter paper inside Petri dishes. The percentage of germinated seeds was counted daily. Germination was evaluated 14 days after sowing. The germination speed index (GSI) was calculated using the formula: GSI = $(G_1/N_1) + (G_2/N_2) + ... + (G_n/N_n)$, where G_n is the number of seeds germinated on day n and N_n is the number of days (Maguire, 1962).

Statistical analysis was performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, IL, USA). The results were analyzed via one-way ANOVA. To determine differences among means, Duncan's Multiple Range Test was employed. A significance level of $p \leq 0.05$ was used to assess statistical significance.

RESULTS AND DISCUSSIONS

The results of this study highlighted the effects of the fertilizers tested on certain quality indices of the round pepper variety 'Asteroid 204' (see Table 2). Significant fluctuations were observed in 1000-seeds weight, fruits per plant, seed yield, seeds per fruit, germination percentage, and germination speed index under the influence of interactions with fertilization methods ($p = 0.000$). The fertilization technique significantly affected all evaluated components, with estimated effect sizes of 67.2% for 1000-seeds weight, 74.2% for fruits per plant, 51.3% for seed yield, 54.4% for seeds per fruit, 81.7% for germination percentage, and 60.7% for germination speed index, according to the ANOVA results.

Table 2. Main effects of fertilization variant and germination days on germination percentage, mean germination time, germination rate index of seeds of the "Asteroid 204" pepper cv.

Evaluated component		1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ Fruit	Germination percentage	Mean germination time	Germination speed index
Fertilization technique	Sig.	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	n.s. (p=0.114)	*** (p=0.000)
	Effect size	67.2	74.2	51.3	54.4	81.7	24.6	60.7

*The symbols and letters of significance refer to the fertilization variant and germination days: n.s. = non significant and
*** = very significant (at $p \leq 0.001$, respectively).

The weight of 1000 seeds ranged from 4.06 to 8.11 g, with an average of 5.23 g. The number of fruits per plant had an average of 4.83, with a minimum of 4.23 and a maximum of 5.31. Seed yield varied between 107.69 and 334.31 kg/ha, with an average of 231.15 kg/ha. The seeds per fruit averaged 791.11 mg, with fluctuations between 552.26 and 1223.90 mg.

The germination percentage was 70.47 ± 3.15%, with minimum and maximum values of 65.00% and 77.00%, respectively. The average germination time was 5.72 ± 0.27 days, ranging from 5.00 to 6.30 days. The germination speed index was 13.20 ± 0.94 seeds germinated/day, varying between 11.66 and 15.43 seeds germinated/day.

Seed germination was monitored from the beginning of the process until its completion. It started on the fourth day after sowing the seeds in Petri dishes and concluded on the eighth day for all treatment types applied. According to Figure 1, the evolution of seed germination over the five days analyzed was variable. The number of germinated seeds decreased on days 5-8 compared to day 4 for the fertilization variants V1 (untreated control), V2 (calcium nitrate), V3 (calcium nitrate and E-Dalgin), and V6 (calcium nitrate and Albit). In contrast, variant V5 (calcium nitrate and Triptolemus HV) showed maximum germination on day 5, while variant V4 (calcium nitrate and Kelpak) reached its peak germination on day 7.

Fluctuations in seed germination behavior were observed, influenced by the six fertilization variants. Variant V1 (untreated control) exhibited the lowest number of germinated seeds on day 5, while variant V4 (calcium nitrate and Kelpak) recorded the fewest germinations on day 6. In variant V6 (calcium nitrate and Albit), the number of germinated seeds decreased continuously starting from day 4, although a slight increase was noted on day 6.

Table 3. Statistical descriptors (mean, median, standard deviation, absolute minimum and absolute maximum) for germination percentage, mean germination time, germination speed index for the pepper cv. "Asteroid 204"

Statistical descriptors	1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ fruit	Germination percentage	Mean germination time	Germination speed index
Mean	5.23	4.83	231.15	791.11	70.47	5.72	13.20
Median	4.99	4.87	230.26	778.21	70.00	5.78	13.14
Mode	4.33a	4.88	107.69[a]	552.26[a]	68.00[a]	5.32[a]	12.39[a]
Std. deviation	0.86	0.27	48.95	130.68	3.15	0.27	0.94
Range	4.05	1.08	226.62	671.64	12.00	1.30	3.77
Minimum	4.06	4.23	107.69	552.26	65.00	5.00	11.66
Maximum	8.11	5.31	334.31	1223.90	77.00	6.30	15.43

[a] Multiple modes exist. The smallest value is shown

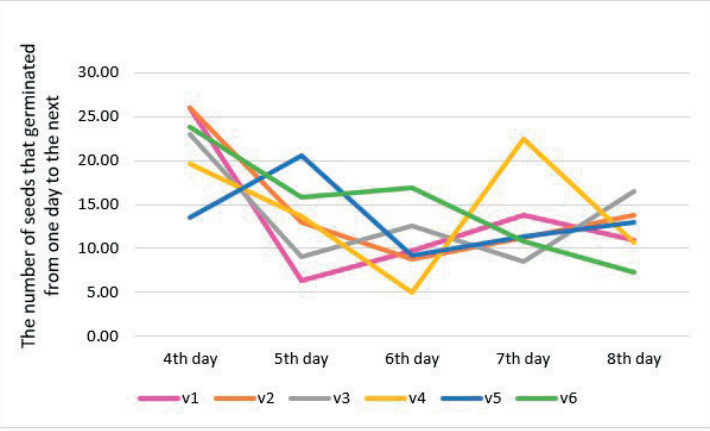


Figure 1. Seed germination evolution (days 4-8) according to fertilization variant

Among the experimental variants studied, the treatments used in variants V4 and V6 led to a significant increase in the weight of 1000 seeds, with the highest values observed in the case of variant V6 (an increase of 32.44%), which involved combined treatments with calcium nitrate and Albit (Table 4). This was followed by variant V4, where treatments with calcium nitrate and Kelpak were applied, resulting in an increase of 21.56%. Previously, increases in 1000 seed weight were obtained in round pepper using calcium nitrate treatments combined with a biostimulant based on *Ascophyllum nodosum* (Buzatu et al., 2025).

The number of fruits per plant increased significantly in all treatment variants compared to the untreated control. The increases ranged from 5.16% (in variant V6, treated with calcium nitrate and Albit) to 15.92% (in variant V4, treated with calcium nitrate and Kelpak). Previous studies have indicated that different biostimulants positively influence the number of fruits in peppers, with significant results observed when biostimulants based on *Trichoderma inoculum* (Constantin et al., 2024; Kumari et al., 2019), *Ascophyllum nodosum* (Buzatu et al., 2025), and *Ecklonia maxima* (Constantin et al., 2024) were used.

Seed yield and the amount of seeds per fruit increased significantly in all treated variants.

The highest seed yield value was obtained in the case of the variant treated with calcium nitrate in combination with the biostimulator Albit. This led to an increase of 62.92%. Similar differences were also observed in the case of the variant treated with calcium nitrate

and the biostimulator Kelpak (V4), in which the increase was 60.94%.

The growth, in the case of the other treatment variants, ranged between 32.55% and 43.36%. Increases in seed yield in round peppers have also been obtained through treatments with calcium nitrate combined with a biostimulant based on *Ascophyllum nodosum* (Buzatu et al., 2025; Constantin et al., 2025).

Regarding the quantity of seeds/fruit, the highest values were also obtained in the case of variants V6 and V4, but significant increases were obtained in the case of all treated variants. The V6 variant led to a difference of 50.16%, and the V4 variant, to a different of 40.71%, compared to the untreated variant V1.

The positive effects of the treatments are due to both calcium nitrate and the biostimulants used. Nitrogen (Aminifard et al., 2012) and calcium (Buczkowska et al., 2016) are essential nutrients for optimal pepper yield. These have a great influence on the round pepper fruit quantity and quality (Constantin et al., 2024). The beneficial effect of seaweed-based biostimulants is due to the high content of phytohormones, amino acids and enzymes (Battacharyya et al., 2015).

Table 4. The influence of foliar treatments on fruits maturated and seeds quantity

Variant	1000 seeds weight (g)	Number of fruits/plant	Seed yield (kg/ha)	Seeds/fruit (mg)
V1	4.87±0.53 b	4.46±0.20 d	165.05±37.30 c	613.31±63.10 c
V2	4.63±0.23 b	4.78±0.12 c	218.78±56.48 b	768.90±69.13 b
V3	4.86±0.25 b	4.87±0.17 bc	231.94±11.51 ab	799.72±49.23 b
V4	5.92±0.38 a	5.17±0.13 a	265.63±21.59 ab	862.99±62.65 ab
V5	4.65±0.42 b	5.02±0.12 ab	236.62±26.49 ab	781.10±62.35 b
V6	6.45±0.99 a	4.69±0.11 c	268.90±47.90 a	920.67±188.32 a

*Duncan test: Significant differences ($p \leq 0.05$) are indicated by mean values in a column that do not share the same letter (a, b, c).

The germination percentage (Table 5) ranged from a minimum of 66.83% (V3, calcium nitrate and E-Dalgin) to a maximum of 74.67% (V6, calcium nitrate and Albit). According to Duncan's test, calcium nitrate and Albit treatment (V6) significantly improved the germination percentage by 5.17% compared to the untreated control (V1), followed by calcium nitrate treatments (V2), which increased germination by 3.33%, and calcium nitrate and Kelpak (V4), which resulted in a 2.00% increase.

A significant decrease in the percentage of germinated seeds was observed after applying calcium nitrate and E-Dalgin or calcium nitrate and Triptolemus HV (V5), with germination rates dropping from 69.50% (V1) to 66.83% (V3) and 67.50% (V5), respectively. The Asteroid 204 pepper variety exhibits superior germination performance, with a percentage of 85% (Șovărel et al., 2023). This performance is influenced by pedoclimatic conditions, the applied technology, the quality of the growing environment, and the level of infestation by pathogens (Hogea et al., 2023).

Previous studies suggest that seed germination performance is significantly influenced by the stage of maturation. Seeds that have reached physiological maturity contain fully developed embryos, giving them an advantage in the germination process compared to seeds at the green maturity stage (Brondo-Ricárdez et al., 2020; Dzib-Ek et al., 2025; Hernández et al., 2020; Sripathy & Groot, 2023) or at the overripe stage, where germination may begin to decline due to increased risks of seed damage (Dos Santos et al., 2016). The seed germination period is favorably influenced by certain substances with a biostimulating role (Dinu et al., 2019).

Environmental conditions such as temperature, humidity, and light also play a crucial role in seed germination. Seeds typically require warm temperatures (between 18-24°C) and adequate humidity to germinate effectively (Bewley & Black, 2013). The average germination time of seeds varies depending on environmental conditions and seed quality (Bewley & Black, 2013; Yazdanpanah et al., 2017). In general, under favorable conditions, germination time tends to decrease.

In the present study, a minimum germination time of 5.49 days was observed for the variant fertilized with calcium nitrate and Albit (V6), closely followed by calcium nitrate alone (V3)

at 5.64 days, and calcium nitrate combined with E-Dalgin (V4) at 5.66 days. In contrast, the control variant exhibited a significantly higher average germination time of 5.81 days.

Table 5. Seed germination behavior

Fertilization option	Germination percentage	Mean germination time	Germination speed index
V1	69.50±0.84c	5.81±0.18a	12.91±0.50b
V2	72.83±1.17b	5.64±0.27ab	13.90±0.67a
V3	66.83±1.60d	5.66±0.32ab	12.73±0.74b
V4	71.50±2.26b	5.87±0.25a	13.03±0.49b
V5	67.50±1.05d	5.85±0.11a	12.25±0.35b
V6	74.67±1.37a	5.49±0.34b	14.39±0.91a

*Duncan test: Significant differences ($p \leq 0.05$) are indicated by mean values in a column that do not share the same letter (a, b, c).

Germination speed index is an indicator of the potential of a seed to germinate. The treatments applied to the seeds also led to significant improvements in the germination speed index (GSI), its value was 1.48 seeds germinated/day higher in the calcium nitrate and Albit-treated variant (V6) than in the untreated control variant (V1). Our results are in agreement with those obtained by Buzatu et al. (2025). Seed sources with a higher germination rate produce stronger seedlings, making them relevant in the selection process for breeding and improving the species (Wani & Singh, 2016).

The intensity of the correlations among the evaluated characteristics, presented in Table 6, indicates that the treatment variant resulted in a significant increase in 1000 seeds weight, fruits per plant, seed yield, and seeds per fruit (r = 0.516**, r = 0.406*, r = 0.613**, and r = 0.620**).

This result suggests that the treatment not only increased the total seed production but also improved the efficiency of each fruit in terms of the number of seeds produced.

Table 6. Correlation matrix between evaluated component

		Variant	1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ fruit	Germination percentage	Mean germination time	Germination speed index
Variant	Pearson Correlation	1	0.516**	0.406*	0.613**	0.620**	0.228	-0.134	0.145
	Sig. (2-tailed)		0.001	0.014	0.000	0.000	0.181	0.437	0.398
1000 seeds weight	Pearson Correlation		1	0.168	0.435**	0.649**	0.502**	-0.156	0.369*
	Sig. (2-tailed)			0.328	0.008	0.000	0.002	0.363	0.027
Fruits/plant	Pearson Correlation			1	0.422*	0.368*	-0.142	0.92	-0.171
	Sig. (2-tailed)				0.010	0.027	0.410	0.595	0.319
Seed yield	Pearson Correlation				1	0.722**	0.250	-0.034	0.141
	Sig. (2-tailed)					0.000	0.141	0.844	0.411
Seeds/fruit	Pearson Correlation					1	0.267	-0.138	0.203
	Sig. (2-tailed)						0.116	0.422	0.235
Germination percentage	Pearson Correlation						1	-0.277	0.789**
	Sig. (2-tailed)							0.102	0.000
Mean germination time	Pearson Correlation							1	-0.794**
	Sig. (2-tailed)								0.000
Germination speed index	Pearson Correlation								1
	Sig. (2-tailed)								

**Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

A positive correlation between 1000 seeds weight and seed yield, seeds per fruit, and germination percentage was also highlighted (r = 0.435**, r = 0.649**, and r = 0.502**). This suggests that seeds with a larger mass may indicate better quality, which can contribute to a higher crop yield.

The relationship between seed yield and seeds per fruit is also positively significant and of very high intensity (r = 0.722**). The result indicates that, in order to improve seed yield, it is important to consider strategies that increase the number of seeds produced per fruit. Germination percentage correlates positively and significantly with the germination speed index (r = 0.789**).

The beneficial interaction between biostimulants, germination rate, and germination speed could enhance crop yields. A very high-intensity negative interdependence was found between mean germination time and the germination speed index (r = -0.794**). This interdependence offers insights into how plants adapt to various cultivation environments, aiding in the understanding of their diversity and survival strategies.

CONCLUSIONS

This study led to the following conclusions:

- treatments with calcium nitrate and Albit led to the lowest mean germination time, meaning a faster germination;
- the highest germination percentages were obtained in the case of variants in which calcium nitrate was combined with Albit, followed by the variants when it was combined with E-Dalgin and Kelpak;
- germination speed index was significantly positively influenced by treatments with calcium nitrate alone or combined with Albit;
- the weight of 1000 seeds was significantly positively influenced by treatments with calcium nitrate combined with Albit or Kelpak;
- the number of seeds per plant, seed yield and seeds/fruit were significantly influenced by all treatments used;
- the use of a biostimulator, in addition to calcium nitrate treatments, leads to better results in seed production and quality.

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STUDY OF THE POSSIBILITIES OF USING COMPOST AS A SUBSTRATE FOR GROWING *BRASSICA OLERACEA* L. VAR. *CAPITATA* L.

Bilyana GRIGOROVA-PESHEVA, Boyka MALCHEVA, Asen PESHEV

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: b.pesheva@ltu.bg

Abstract

The present study aimed to trace the change in microbial communities and the degree of cabbage formation under different variations of the growth medium. Five variants were studied: control - soil, V1 - 10% compost and 90% soil, V2 - 25% compost and 75% soil, V3 - 50% compost and 50% soil, V4 -100% compost. Samples were taken on 15, 30 and 60 days. The amount of total microflora in all variants slightly decreased over time, but the mineralization rate remained almost the same. Throughout the experiment, the main share in the composition of the total microflora was occupied by non-spore-forming bacteria and bacillus, and actinomycetes and micromycetes were less represented. The total amount of microorganisms was higher in the variants with compost compared to the control, highest in the variant with 100% compost content.

Key words*: microorganisms, compost, Brassica oleracea L. var. capitata L.*

INTRODUCTION

Composting converts raw organic materials into a biologically stable, humic-like product called compost (Ceglie & Abdelrahman, 2014; Graves and Hattemer, 2000). Compost mixtures with an optimal carbon/nitrogen ratio of around 30:1 of the input materials shows a higher microbial number and better physicochemical characteristics of the finished product. (Grigorova-Pesheva et al., 2024). Given the deteriorating state of soils across Europe, both in terms of physical characteristics and nutrient content (Ferreira et al., 2022; Lawrence & Melgar, 2023), the possibility of using compost as a basis for growing certain crops is of interest for developing agriculture and meeting the needs for ever higher productivity. The composition and quality of the finished compost varies greatly depending on the starting substrates used in the composting process. (Miyamoto et al., 2023). Furthermore, composting is essentially a biological transformation of organic matter under the action of microorganisms (Rastogi et al., 2020). Different groups of microorganisms participate in the composting process, and their distribution and abundance affect the quality of the finished product (Silva & Naik, 2007). As a result, when using compost as a base for seedlings, the characteristics of the finished compost, its organic matter content, fractional composition, as well as acidity, have a strong influence on the development and formation of crops (Chaudhuri et al., 2016). It is for this reason that the present study aimed to evaluate different combinations in percentage terms of compost and soil. The production of vegetable seedlings worldwide is enormous. As noted by Carmona et al. (2012) in their article, given the limited volume of seedling containers, the quality of the substrate is of key importance.

In their study, although related to decorative plants, the team of Giuffrida (2017) found that certain compost mixtures can completely or partially replace the soil substrate used until now. This suggests the possibility of using certain compost mixtures as an alternative to soil substrate, which will lead to significant economic benefits for agriculture and mainly for vegetable production.

In this study, the experiments were carried out with cabbage (*Brassica oleracea* L. var. *capitata* L.), given the large percentage it occupies in vegetable production in Bulgaria. In addition, cabbage crops are classified as undemanding in terms of soil type, but are particularly demanding on soil fertility

(Daskalov & Kolev, 1958). Namely because of this, cabbage is a suitable test subject for transplanting in containers with different variants of soil-compost mixture. Moreover, given the low acidity levels preferred by cabbage seedlings (Daskalov & Kolev, 1958), as well as the need for high levels of organic matter (Kolev, 1963; Manolova, 2012), the soil-compost mixture appears to be a potential alternative for their production, including as a lower budget option. The great economic importance of cabbage has also led to various scientific developments related to its cultivation in combination with other crops. A comparative study of root and soil microbiological activity in individual and intercropping cultivation of cabbage (*Brassica oleracea* L. var. *capitata* L.) was carried out, and the influence of different mixed crops on the microbial abundance of the cabbage rhizoplane was also studied. (Mladenova et al., 2023; Mladenova et al., 2024a; Mladenova et al., 2024b).

The main goal of this study is to investigate the potential for using different variants of soil-compost mixtures for growing cabbage seedlings, based on microbiological indicators, as well as on the degree of seedling development.

MATERIALS AND METHODS

In our study, cabbage was used as an experimental seedling. Cabbage is a major representative of the Brassicaceae family, an extremely commonly used vegetable in Bulgaria, which is also very suitable for intercropping (Mladenova & Yordanova, 2023). For the purpose of this study, cabbage was also chosen, given that it ranks second in production in Bulgaria for 2023 with 44,963 tons per year. (Ministry of Agriculture and Food, Bulgaria, report 437, 2024). For the experimental part, five variants were prepared: control - soil, V1 - 10% compost and 90% soil, V2 - 25% compost and 75% soil, V3 - 50% compost and 50% soil, V4 - 100% compost. Mature compost obtained from the transformation of potatoes, wood chips, green leafy vegetables and starter soil in a ratio of 20/2.5/10/7.5 was used, with the initial C:N ratio of 31:1. The compost was previously analyzed for the presence of pathogens above the maximum permissible levels, which were not detected. The finished compost used in this scientific work has the following characteristics: C:N - 14:1, pH - 7.2, suitable structure of the formed aggregates, N - 1.76%, C - 24.64%. The soil used is from the Vrazhdebna farm, where the experiment was conducted. The soil has the following characteristics: $pH_{(H_2O)}$ - 6.9, organic carbon (Corg.) - 1.72% P_2O_5 - 15.96 mg/100 g, K_2O - 22.91 mg/100 g, N - 0.11%.

In each of the variants, seedlings of *Brassica oleracea* L. var. *capitata* L. were planted. The microbiological status of the different mixtures and the control was studied, as samples were taken on 15, 30 and 60 days. The amount of *Bacillus*, non-spore-forming bacteria, micromycetes and actinomycetes was determined. For the determination of *Bacillus*, the samples were previously pasteurized, with subsequent inoculation on Nutrient agar at 24°C for 48 hours. For non-spore-forming bacteria, nutrient agar was used, and the cultivation was done at 24°C for 48 hours. The cultivation of actinomycetes was carried out on Starch ammonia agar at 35°C for 7 days, and of fungi on Čapek doxa agar, for 7 days, at 30°C. Based on the quantities of individual groups of microorganisms, the total microbial count was determined. Each inoculation was repeated five times, and the average value was given. The results are presented in column-forming units (CFU) x 10^5 per gram of absolutely (abs.) dry mass. In addition to the microbiological parameters, the external signs of development of the product part - cabbage - were also examined. The only treatment applied was watering.

RESULTS AND DISCUSSIONS

The results of the experiments are presented in CFUx10^5/g dry mass. The reported values for microbial abundance expressed as the total microbial number of the individual variants by day are presented in Figure 1.

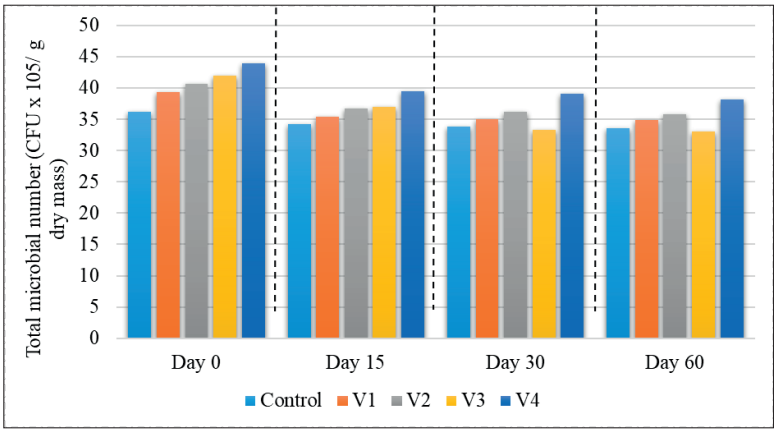


Figure 1. Total microbial number (CFU*10^5/dry mass)

The data from analyses of the total microbial number show a slight decrease in microbial abundance as time progresses. At the beginning of the experiment (day 0) the highest total amount of microorganisms was found in the compost sample (V5). The lowest number of microorganisms is expected for the soil sample (100% soil, V1). In the other variants, as the percentage of compost increases, the amount of microflora also increases. Given that the increased amount of microflora leads to a faster transformation of organic components into forms suitable for plants (Chen et al., 2024), we believe that variants with a higher percentage of compost will provide a greater amount of nutrients to the grown seedlings. As time progresses, microbial abundance in all variants decreases. The difference between day 0 and day 15 is the largest. Between day 30 and day 60, almost no difference is observed, as the system has reached equilibrium. Compared to the baseline data, microbial abundance decreased most significantly in V4, which is equal parts compost and soil. The reduction in total microbial number compared to the baseline data is as follows: Variant 1: 7.34%, Variant 2: 11.39%, Variant 3: 11.84%, Variant 4: 21.35% and Variant 5: 13.06%. Similar data show the specificity of using soil-compost mixtures. The main risk is the reduction of microbial abundance, respectively the reduction of the transformation of the available organic matter. Thus, in the initial sample with only soil, the microorganisms decreased almost two times less compared to the sample of simple compost (V5). Of interest are the data that show a higher decrease in microbial abundance with increasing percentage of compost, as in sample 4, the total microbial number decreased by 21.35%. More than in the variant with compost (V5). Apart V5, which is just compost, V3 stands out (compost 25%, soil 75%) which has the highest total number of microbes.

Figures 2-5 show the quantities of individual microbiological groups in the studied samples in dynamics.

The data on the studied quantities of individual microbiological groups show a dominant presence of the group of non-spore-forming bacteria regardless of the variant and the number of days of reporting. Given that, these are the microorganisms that predominate in the final phases of organic matter transformation, the data show a stable state of the compost mixtures and similar microbiological characteristics to the soil.

After non-spore-forming bacteria, the most common is the bacillus group, followed by the micromycetes and actinomycetes groups, which are almost equally represented.

The data from the microbiological analyses on day 15 show a slight change in the distribution of microbial groups. Thus, the amount of non-spore-forming bacteria decreases slightly at the expense of the slightly increased amount of *Bacillus*. In all samples, an increase in the amount of micromycetes compared to the amount of actinomycetes was observed.

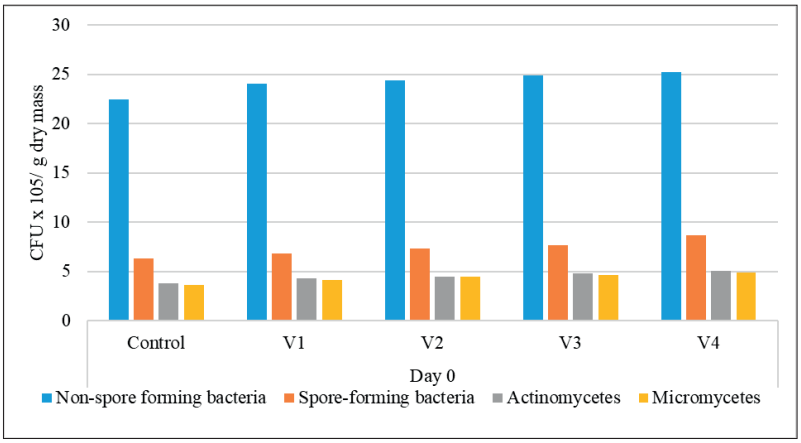


Figure 2. Microbial number of main microbial groups at Day 0 (CFU*10^5/ dry mass)

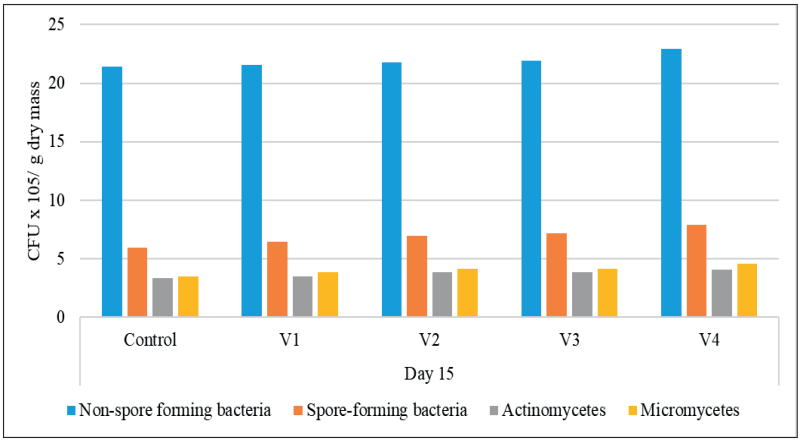


Figure 3. Microbial number of main microbial groups at Day 15 (CFU*10^5/ dry mass)

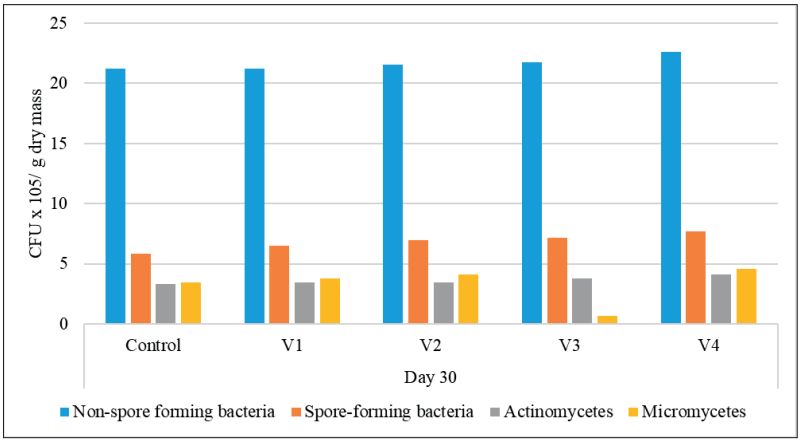


Figure 4. Microbial number of main microbial groups at Day 30 (CFU*10^5/ dry mass)

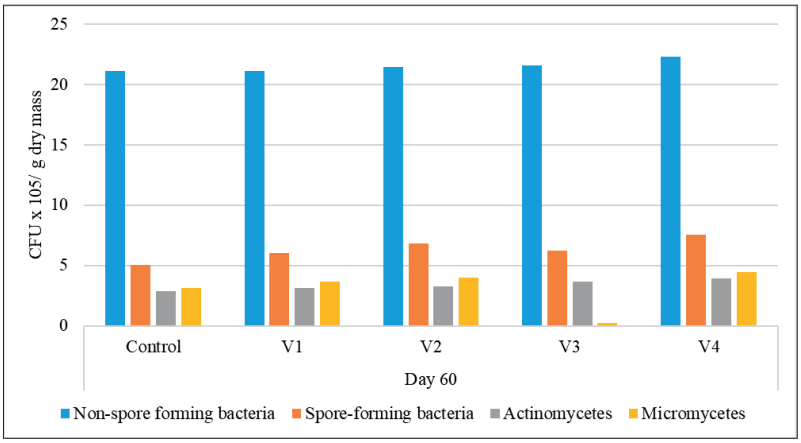


Figure 5. Microbial number of main microbial groups at Day 60 (CFU*10^5/ dry mass)

The values reported for day 30 show the occurrence of certain changes in V4. In the other variants, there is no significant dynamics in the results. In V4, the amount of fungal microflora has greatly decreased, constituting only 6% of the total microflora. In the other variants, this percentage is around 27%. Similar data indicate a certain suppression of the fungal microflora, probably as a result of the greater participation of the compost mass, in which there is a specific development of microorganisms that can exhibit a certain competition with the soil ones. There are studies that show that when materials are mixed, microorganisms that are autochthonous to both mixtures appear allochthonous to the mixed material, which leads to a certain competition between the groups, which continues until equilibrium is reached, often by displacing one species over the other (Boehme et al., 2004).

The data on the microbiological characteristics at day 60 maintain the trends from the previous samplings. However, in V4 we observed an almost complete absence of the fungal group. In this variant, when mixing the two starting materials used, specific settling in places and non-formation of a soil-like structure of the mix were observed. We assume that the increased compost content leads to a certain change in the formation of the structural aggregates of the mix, which changes the water-air regime. These data are also explained by the higher levels of anaerobes reported in this variant. This is probably one of the reasons for the strong reduction of the fungal microflora, which is known to be highly aerophilic.

During each reading of the microbiological parameters of the seedling production variants, the external development of the cabbage seedlings used was observed. For the purpose of the experiment, equally developed seedlings were introduced on day 0. In order to track their development and the potential development of cabbage, the tracking was carried out within 60 days. At each sampling, the development of the stem and leaf mass was recorded. At the beginning, the variant with 100% compost showed much faster stem development and leaf formation compared to the other variants. The development of the stem was about 37% greater. The variant with 100% soil (V1) also had very good initial development. It is interesting that at the initial reading on day 15, V2 (10% compost, 90% soil) stood out with the weakest development.

Measurements on day 30 showed that all variants had similar characteristics, with a delay in the development of Variant 5, with pure compost. Development and formation of cabbage was observed in all variants. On day 60, the data indicated that the variant with pure compost had the weakest development of cabbage, despite the initial rapid growth. Variant 2, with 25% compost and 75% soil, stood out with the best-formed and fresh cabbage. Similar data showed that soil-compost mixtures are suitable not only for growing cabbage seedlings, but also for the entire vegetative cycle. Given the generated results,

we believe that when building appropriate beds with a base of soil-compost mixture, the entire cultivation until harvesting of cabbage can be carried out on them.

CONCLUSIONS

The present study was conducted to investigate the possibility of using compost as a potential substrate for the development of *Brassica oleracea* L. var. *capitata* L. The present work examined four variants of compost mixture and one control. For each of the variants, the total microbial count and the amount of individual microbial groups were determined at the beginning of the experiment, on day 15, day 30 and day 60. The state of seedling development was also assessed. As time progressed, a decrease in the total number of microorganisms in all samples was found. In all samplings, V5, with only compost, and V3, with 25% compost and 75% soil, was characterized by the highest microbial abundance. For all variants, the group of non-spore-forming bacteria predominated. In general, the variants have similar microbiological characteristics, with the exception of V4. In this variant, a strong suppression of the fungal microflora was observed, which almost disappeared at the end of the experiment.

When analyzing the external signs of the seedlings on day 15, its more rapid development is clearly evident in V5, with compost. After it, the best development is observed in V1 (100% soil). On day 30, the seedlings have almost similar signs, which emphasizes the slowdown in the rapid development of V5. At the end of the experiment, V3 stands out with the best development, which has the best-formed cabbage.

The present experiment proved that the use of a soil-compost mixture is suitable for growing cabbage seedlings.

Such a way of growing seedlings would reduce financial costs, especially for mixed farms that generate large quantities of compost. However, it is important to take into account that each compost has specific characteristics in relation to the starting materials used.

Based on the conducted research, we recommend that if rapid seedling development and subsequent sowing is required, the variant with 100% compost or with 25% compost should be used. However, if the seedling is kept for a longer period of time, the variant with 100% compost is not applicable and it is necessary to resort to the option with 25% compost. In all cases, we recommend using compost mixtures when growing cabbage, even in open areas, given this crop's need for a sufficient amount of organic matter in the environment.

This study can serve as a basis and foundation for future in-depth research on the topic. The findings of this study represent a basic platform for the future use and utilization of compost mass generated on farms.

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STUDY ON THE IMPACT OF ELECTROMAGNETIC FIELDS ON THE GROWTH AND DEVELOPMENT OF CERTAIN VEGETABLE PLANT SPECIES

Roxana Ioana GABOR ILIESCU, Elena DOBRIN, Adrian PETICILĂ, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author emails: draghiciem@yahoo.com, elena.dobrin@gmail.com

Abstract

Electromagnetic fields can influence plant growth and development, with their effects depending on frequency, intensity, and exposure duration. Research conducted so far has highlighted both positive and negative effects. Regarding the beneficial effects, exposure to low-frequency fields has been associated with faster seed germination, stimulation of stem and root growth, and increased biomass accumulation. Additionally, these fields can activate metabolic processes by increasing the levels of plant hormones (auxins, gibberellins) and improving enzymatic activity, supporting the overall health of plants. On the other hand, prolonged or intense exposure can lead to negative effects, such as oxidative stress caused by an excess of reactive oxygen species (ROS), which can damage cells. Some studies show growth inhibition, manifested by reduced root and stem length, as well as a decrease in chlorophyll content, which affects photosynthetic efficiency. The aim of this study is to analyze the impact of different wavelengths on the growth of vegetable plants, in order to identify optimal electromagnetic conditions that could enhance agricultural productivity and reduce associated risks.

Key words*: electromagnetic fields, seeds, germination.*

INTRODUCTION

The study of the impact of electromagnetic fields on the growth and development of certain species of vegetable plants is a topical and highly interesting topic in the context of increasing exposure to electromagnetic radiation from various sources. The results of existing research indicate that these fields can have variable effects on plants, influencing essential physiological processes, such as seed germination, root growth, plant growth, photosynthesis, and water and nutrient absorption.

These physical methods are ecological and influence the physiological and biochemical processes of rice seeds (Carbonell et al., 2000) and onion García et al. (2002) leading to faster germination and improved plant development.

Some studies have shown positive effects, such as stimulating growth and biomaterial production, while others have reported inhibitions, oxidative stress and changes in plant cell structure (Iderawumi & Friday, 2020). Maffei (2014) and Pawełek et al. (2022) state that exposure of seeds and plants to static magnetic fields and non-ionizing electromagnetic fields showed positive effects on germination, growth rate, stress resistance and yield.

MATERIALS AND METHODS

The study analyzes the influence of electromagnetic fields on seed germination. It examines the impact of magnetic fields on seed germination, plant growth, and fruiting.

RESULTS AND DISCUSSIONS

Shabrangy et al. (2021) and Shabrangy (2024) investigated methods for using different magnetic fields to improve seed germination, plant growth, and yield. They specify that these methods include magnetic fields weaker than the geomagnetic field, reversed magnetic fields, strong static magnetic fields, electromagnetic fields, magnetized water, and high-gradient magnetic fields.

The effect of the electromagnetic field on seed germination. has been noted by numerous researchers. Etimad et al. (2022) mentions in their study that magnetized water, obtained by passing it through a special magnet, can stimulate plant growth and productivity. It also improves germination, vegetative development, nutrient absorption and photosynthesis, while reducing soil salinity. At the same time, they conclude that the electromagnetic field can transform poor quality water into a useful resource.

Naz et al. (2012) stated that the effect of pre-planting magnetic treatments on germination, growth and yield of okra (*Abelmoschus esculentus* cv. Sapz paid) by exposure to a magnetic field of 99 mT for 3 and 11 minutes led to a significant increase ($P < 0.05$) in germination, number of flowers, leaf area, plant height, number of fruits and seeds, and fruit mass per plant. They concluded that exposure to the value of 99 mT for 11 minutes had the best results compared to the control.

Experiments conducted to see the influence of electromagnetic field intensity on okra seed germination indicated that both the seed exposure time and the electromagnetic field frequency had a favorable effect on germination time and the percentage of germinated seeds (Rachieru et al., 2022).

Antonova-Karacheva (2021) based on the results obtained showed a positive effect obtained in seeds treated with a voltage of 6 kV, exposure of 20 seconds and an interval of 8 days until sowing.

Ziaf et al. (2022) claim that treatment of okra seeds with 100 mT magnetic fields for 20 minutes improved germination, growth and plant yield. Also, priming of seeds in 1% KNO3 solutions increased chlorophyll content and photosynthetic efficiency. These methods can increase the efficiency of agricultural production.

Souza Torres et al. (1999) exposed tomato seeds of cv. Campbell 28 to magnetic field values of 0.08, 0.10 or 0.17 Tesla for 1-25 minutes. 18 variants of the 21 treatments showed an improvement in germination after 5 days, and 10 variants after 7 days. They noted that the growth of seedlings was 5-25% in the case of 12 treatments, the best result being obtained with 0.10 T for 10 minutes. It was found that 16 treatments favored the increase in root length.

The study by Charles et al. (2021) aimed to investigate the effects of microwaved water on the growth of tomato seedlings (*Lycopersicon esculentum*). They used three variants: seeds and plants treated with tap water, boiled and cooled water, and microwaved water, for 5 weeks. The results showed that both boiled and microwaved water stimulated stem growth, with the greatest positive effect observed for boiled water. This study suggests that microwave radiation did not have a negative impact on plants and that the use of microwave appliances does not negatively affect the heated substances or human health.

Peteiro-Cartelle and Cabezas-Cerrato (2009) analyzed the influence of static magnetic fields on mitosis in *Allium cepa*. They found that the magnetic field did not modify the mitotic index, but increased the prophase and metaphase indices, while decreasing those of anaphase and telophase. An increase in the rate of cell emergence was also observed, and cytogenetic effects were minimal. The results indicated a shortening of the duration of anaphase-telophase under the influence of the magnetic field.

Jeong et al. (2024) found that the application of a directed electromagnetic field stimulated the germination and growth of radish plants.

Katsenios et al. (2021) in an experiment conducted on kale and spinach seeds exposed to a pulsed electromagnetic field at three growth stages found that some treatments increased plant weight, positively influencing growth characteristics.

Exposure of radish seeds to stationary magnetic fields stimulated germination and vigor, with optimal effect at 20 mT for 12 minutes, while the variable field for 12 minutes had a negative impact (Konefał-Janocha et al., 2019).

Kaigorodova et al. (2021) in their experimental work showed that in the ranges of 5-440 B/m (electric) and 0-0.53 μT (magnetic), the electromagnetic field stimulated the growth of beans, increasing chlorophyll (15-65%), polyphenols (17%), antioxidant activity (1-15%) and ascorbic acid (12-28%). They observed a reduction in carotene at 60-100 B/m and the lack of correlation between pigments and antioxidant activity at technical maturity.

Applying an 88 mT magnetic field in a sequencing batch reactor enhanced biomass aggregation (75.0-95.2%) compared to the control (65.5%), reducing sludge volume index (25.8 ml/g vs. 73.1 ml/g) and improving COD and ammonia-nitrogen removal. This suggests that magnetic fields can mitigate sludge bulking and enhance pollutant removal efficiency (Zaidi et al., 2022).

Krylov and Tarakonova (1960) discovered the effects of magnetic fields on plants, suggesting an auxin-like effect called magnetotropism. Boe and Salunkhe (1963) associated it with tomato ripening. It was found that roots respond more to magnetic fields than to light, and species-specific factors are essential for the manifestation of tropism (Pittman, 1962).

Haq et al. (2012) reported a reduction in germination time under different magnetic field intensities, both under alternating current (AC) and direct current (DC) conditions. They observed a significant decrease in germination time only at 110 mT for 10 minutes.

Shine et al. (2011) conducted experiments on soybean seeds (Glycine max) using static magnetic fields and found that germination, seedling growth and biomass were improved, with the results being maximal at 200 mT (60 min) and 150 mT (60 min). These treatments increased photosynthetic efficiency and leaf protein content, demonstrating significant benefits for plant growth.

Moon and Chung (2000) cited by Iderawumi and Friday (2020) state that treating tomato seeds with AC electric fields with intensities ranging from 4 to 12 kV/cm and AC magnetic flux densities ranging from 3 to 1000 G for 15 to 60 seconds accelerated the germination rate by approximately 1.1-2.8 times compared to untreated seeds. However, an inhibitory effect on germination was observed when the electric field exceeded 12 kV/cm or the exposure time was greater than 60 seconds.

Studies by Mukhammadiev et al. (2022) have shown that electrical stimulation of potato tubers before planting can accelerate shoot emergence by 3-4 days, depending on the variety. Electrically stimulated plants also showed more vigorous growth, being 4-5 cm taller and having a greater number of stems. In a second experiment, each electrically stimulated bush produced an average of 813 grams of tubers, compared to 398 grams in the control group. Visual and serological analyses indicated that electrical stimulation reduced viral infections in plants. For example, the Kuvonch-1656M variety showed an infection rate of 12% in the studied variant, compared to 16% in the control variant, and latent viral infections were 26% and 31%, respectively.

Corey et al., (2013) mention that plants produce a variety of hormones that regulate growth, development and response to environmental stress. In addition to the perception and signaling of these molecules, the enzymes that control their dosage and activity are also essential. The scientific study explores the role of enzyme families, such as SABATH methyltransferases, methylesterases, GH3 acyl acid-amido synthetases and peptide hydrolases, in the biosynthesis and modification of hormones, contributing to the chemical signaling network that supports plant adaptation.

Etimad Alattar, Eqbal Radwan (2020) conducted a study investigating the effect of water treated with radiofrequency electromagnetic radiation on the growth of pepper plants (*Capsicum annuum*). The plants were divided into two groups: one watered with water exposed to radiation from a Wi-Fi router for one hour per day and one with tap water (control). The results showed that the treatment had a negative impact on growth, causing a reduction in shoot length, stem diameter, root length and plant health index. There were also decreases in the number of leaves, flowers, fruits and seeds, as well as a significant delay in flowering.

Ramezani et al. (2012) in their study stated that electromagnetic radiation can be a stress factor for plant development, influencing metabolism, gene expression and growth. The effects occurred both in directly exposed and adjacent tissues, being influenced by root characteristics and plant condition. 14.3 Hz magnetic fields can protect plants from drought, delaying harmful changes in transpiration and photosynthesis. Under normal conditions, no significant effects were observed, except for a decrease in relative transpiration.

Sincak et al. (2023), in their study affirms that the electromagnetic fields offer a promising method for controlling biotechnological

processes, influencing cell growth and metabolism without altering the culture medium or the resulting products. The analysis of 103 experimental and theoretical studies highlighted beneficial effects such as accelerated microbial growth, stabilization of bacterial communities in wastewater treatment, improved fermentation, and enhanced metal bioleaching. Although these applications are promising and sustainable, the use of magnetic fields in biotechnology remains largely unexplored.

Nagy and Fischl (2004) reported that a static magnetic field (0.1-1 mT) reduced fungal colony growth by 10%, with significant effects at 0.1 mT (P = .001). Conidia production increased (68–133%) in *Alternaria alternata* and *Curvularia inaequalis*, but decreased (79-83%) in *Fusarium oxysporum*, with generally significant deviations (P = 0.05).

Magnetic treatment of irrigation water improved productivity and water savings for eggplant, faba beans, and tomatoes, reducing soil pH and increasing crop yield. Water savings ranged from 11% to 14.2%, and profitability increased significantly (Nessrien, 2018).

Basant and Harsharn Singh (2009) conducted a study on the magnetic treatment of different types of irrigation water and its impact on the productivity of snow pea, celery, and green pea plants. The experiments were carried out in greenhouses using potable water, recycled water, and saline water treated magnetically. The results showed significant increases in yield and productivity for celery and green peas, particularly when recycled water and 3000 ppm saline water were used. However, no positive effects were observed on pea plants. Additionally, the magnetic treatment of water had an impact on the soil, reducing pH and increasing electrical conductivity and available phosphorus in the soil for celery and green peas.

CONCLUSIONS

Exposure to electromagnetic fields positively influences the growth rate of vegetable species, leading to greater height, leaf area, and biomass accumulation.

Seeds subjected to electromagnetic field treatment had higher germination rates and sprouted more quickly compared to untreated seeds.

Crop yield increase. Exposure to electromagnetic fields resulted in higher yields, with significant improvements in the size, weight, and productivity of the fruits.

Regarding water and nutrient absorption, it was found that treated plants showed higher efficiency in absorbing water and nutrients, contributing to better resistance to environmental stress.

Studies revealed certain physiological changes, including increased enzyme activity, chlorophyll content, and photosynthesis rates, suggesting enhanced metabolic activity under the influence of electromagnetic fields of certain intensities.

Regarding species-specific responses, it was found that the effects varied depending on the vegetable species, with some plants responding more positively than others.

Scientific results suggest that controlled exposure to electromagnetic fields could be a promising technique for improving vegetable production while reducing resource inputs.

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COMPARATIVE STUDY OF INTRODUCED SWEET CORN VARIETIES

Neli KERANOVA[1], Kostadin KOSTADINOV[1], Nadezhda SHOPOVA[2], Radoslav CHIPILSKI[3]

[1]Agricultural University of Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria
[2]Climate, Atmosphere and Water Research Institute at Bulgarian Academy of Sciences (CAWRI-BAS), 66 Tzarigradsko Blvd chaussee, 1784, Sofia, Bulgaria
[3]Institute of Plant Genetic Resources, 2 Drouzhba Street, 4122, Sadovo, Plovdiv District, Bulgaria

Corresponding author email: kostadinov8888@gmail.com

Abstract

Sweet corn is a crop highly sensitive to climatic conditions, which raises scientific interest in its productive indicators under climate change conditions. This study presents a comparative analysis of four promising hybrids for Bulgaria – Zeaton F1, Turbo F1, HMX5389 F1, and HMX59YS832 F1. The experiments were conducted in 2023 and 2024 on the territory of the Agricultural University of Plovdiv. The results show significant differences between the hybrids, with Zeaton F1 exhibiting the best biometric indicators and a maximum plant height. Plant development and especially critical periods coincided with stressful summer conditions. The second year was characterized by more extreme climatic events, but late sowing reduced the negative impact on yield. It was established that growing sweet corn in the dry conditions of Plovdiv is impossible without irrigation, while drip irrigation significantly improved moisture reserve. Hybrids with a shorter growing season showed better growth parameters when sown late. A strong correlation was observed between plant height and the number and length of leaves.

Key words: *productivity parameters, sweet corn, meteorological conditions, biometric indicators*.

INTRODUCTION

Sweet corn is significant nutritionally and economically. Numerous studies highlight its dual role as a nutritious food source with health benefits and as a commercially valuable crop in the agricultural and food industries. Sweet corn is a specialized type of maize, distinguished by its high sugar content during the milk stage (Singh et al., 2014). It is widely cultivated for fresh consumption, canning, and freezing, making it a key component of the vegetable industry. Additionally, sweet corn plays a crucial role in producing natural sweeteners, which serve as alternatives to synthetic sugars, thereby contributing to the food processing sector. The crop's market demand and adaptability to different agro-climatic conditions further establish its profitability.

The nutritional benefits of sweet corn position it as a future staple in human diets. Swapna et al. (2020) highlight its rich content of essential nutrients, including vitamins, minerals, and antioxidants, which contribute to improved digestion, eye health, and chronic disease prevention. Its tenderness and taste make it a versatile ingredient in various culinary traditions. Furthermore, the development of new varieties is essential to meet growing consumer demand and enhance its nutritional quality.

Agricultural factors play a critical role in determining the yield and quality of sweet corn (Sidahmed et al., 2024). Beyond its high sugar content and tender kernels, sweet corn is valued for its broad market appeal. Factors such as temperature, soil fertility, irrigation practices, and nutrient management directly influence its growth, development, and yield potential. Selecting varieties suited to specific agro-climatic conditions is essential for sustainable production. Additionally, advancements in agronomic techniques can lead to improved crop performance and greater profitability for farmers. Global research efforts have significantly advanced sweet corn cultivation in recent years. Revilla et al. (2021) discuss the crop's significance as a nutrient-rich food source containing essential vitamins, minerals, and antioxidants beneficial for human health. Innovations in breeding techniques have improved disease resistance, stress tolerance, and nutritional content. Research also addresses

challenges posed by biotic and abiotic stresses, such as pests, diseases, and climate variability, with modern agricultural practices and genetic improvements mitigating these issues. The growing global demand for sweet corn is driven by consumer preferences for healthier food options and its versatility in fresh and processed forms.

Recent studies provide valuable insights into sweet corn improvement. Revilla, Anibas, and Tracy (2021) offer a comprehensive review of research from 2015 to 2020, emphasizing genetic advancements, breeding strategies, and crop management innovations. Ziqi Li et al. (2024) explore the nutritional quality of sweet-waxy corn across three regions in China, demonstrating the influence of local environmental conditions on varietal responses. Tracy, Shuler, and Dodson-Swenson (2020) focus on genetic improvements, particularly the role of endosperm genes in enhancing kernel sweetness and texture.

Evaluations of vegetative and physiological traits have provided insights into the adaptability and productivity of sweet corn varieties. Elayaraja et al. (2018) emphasize the importance of combining ability and genetic factors in influencing growth, yield, and quality in hybrid sweet corn. Similarly, Tracy et al. (2020) examine physiological responses, such as photosynthesis and stress tolerance, offering guidance for breeding programs and agronomic practices. Collectively, these studies underscore the need to identify superior varieties with enhanced vegetative and physiological performance for sustainable cultivation across diverse ecological zones.

A synthesis of recent research underscores advancements in genetic improvements, market trends, and crop management strategies (Revilla et al., 2021). Studies demonstrate how regional environmental conditions influence the nutritional quality of sweet-waxy corn (Ziqi Li et al., 2024) and provide insights into varietal differences, physiological behaviours, and environmental interactions essential for sustainable production (Elayaraja et al., 2018; Tracy et al., 2020). Investigations into the role of endosperm genes continue to enhance sweetness and texture.

Building on these findings, this research aims to contribute to the broader knowledge of sweet corn improvement. The outcomes will support sustainable cultivation practices, inform breeding strategies, and aid in developing superior genotypes, benefiting stakeholders across the agricultural value chain. By analyzing growth parameters, photosynthetic efficiency, and stress tolerance, this study seeks to identify genotypes with enhanced adaptability and productivity. Integrating recent literature with field-based assessments, this work bridges scientific research and practical applications to advance sweet corn cultivation.

MATERIALS AND METHODS

The experimental work was conducted in the period 2023-2024 in the field of AU-Plovdiv with four sweet corn hybrid varieties: Zeaton F1, Turbo F1, HMX5389 F1 and HMX59YS832 F1. The plants were sowed according to the scheme 70/20 cm on 1 June. The experiment was carried out according to the block method in 4 variants with four repetitions, with 50 plants per repetition and the size of the experimental plot 7 m^2. Watering was carried out with a drip system. The following variants were tested: 1. Zeaton F1 - Control; 2. Turbo F1, HMX5389 F1; 3. HMX5389 F1; 4. HMX59YS832 F1. The plants were fertilized with Gold Forte-organomineral fertilizer containing N 48%, P 8%, K 4%, Mg 2%, Mn 0.4%, Mo 0.02%, Zn 2%, Free acids 6%, Alginic acid 0.2%, Gibberellic acid 150, pH: 4.5-6.5. Fertilization was applied at a rate of 1000 ml/da, four times in vegetation- 2-leaf stage, 6-leaf stage, before tasselling, and during grain filling.

By conducting biometric measurements, the vegetative behaviour in the consumptive maturity of 12 plants of the variant was monitored. The following indicators were recorded: plant height (cm); number of leaves per plant; leaf length (cm); leaf width (cm); height of ear placement (cm); number of branches per tassel; Ear length (cm); ear diameter (cm):

Meteorological data. For this study, an automatic meteorological station was installed in the experimental field. During the potential growing season, the following meteorological parameters were measured: average, maximum and minimum air temperature (°C), relative air

humidity (%), and daily precipitation, (mm). The plants were cultivated under drip irrigation conditions.

A non-invasive field screening of four corn hybrid varieties was performed by leaf chlorophyll content index (CCI) measurement with device CCM200+, manufactured by Opti Science UK.

The data were analysed using one-way analysis of variance (ANOVA), Fischer's least significant difference (GD) test, and correlation analyses.

RESULTS AND DISCUSSIONS

The measured meteorological values give reason to define 2024 as one of the warmest years since the beginning of meteorological observations in the region. A lower average temperature is observed before seed sowing, with the only negative deviation in 2024 being in May (Figures 1 and 2). Hydrothermal conditions for sweet corn cultivation are favourable after mid-April. However, for testing stress factors according to the set objectives of the experiment, sowing was carried out in early June. During the initial period of corn development - June, the average temperature in 2024 is 4.1°C higher than in 2023.

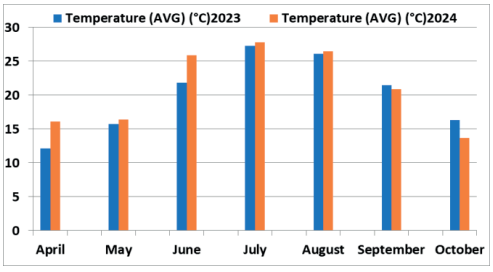


Figure 1. Average air temperature by month for 2023 and 2024

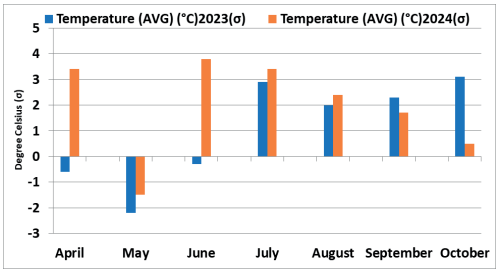


Figure 2. Deviation in average monthly air temperature from the climate norm 1991-2020

The number of days required for sweet corn seeds to germinate strongly depends on the average soil and air temperature. In June 2023, temperatures above 20°C combined with optimal soil moisture allowed germination to occur in about 5 days, and even higher average temperatures in 2024 led to seed germination within just a few days. The total vegetation period for Zeaton F1 suggests 78-80 days after germination, while for the Turbo F1, it is 8-10 days less. Under the conditions of the country, the period from leaf to leaf requires effective temperatures of about 30-32°C, and in dry and hot weather this amount increases to over 42°C (Hershkovich, 1975). During 2023, the plants entered the reproductive phase after 40 days, while in the warmer June of 2024 after 30 days. The duration of the critical phase of tasselling-silking is about 17 days and the period to milk maturity-10 days. In conclusion, with this later sowing, the critical periods for the plants are in the second half of July and at the beginning of August.

The second experimental year was significantly drier than 2023 (Figure 3). However, during the sowing period and leaf formation, precipitation in 2024 was higher than that in 2023.

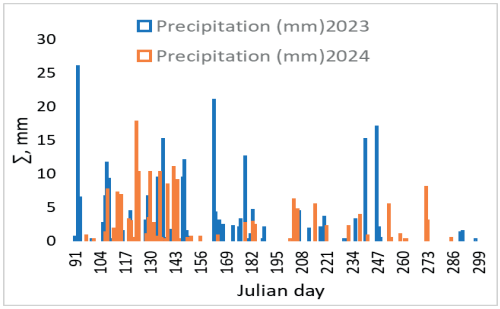


Figure 3. Precipitation amount (mm) by day in 2023 and 2024

The collected meteorological data show that the maximum temperatures in 2024 are also significantly higher compared to those in 2023 (Figure 4). In the first experimental year (2023), unfavourable conditions began in the third decade of June, with 54 days with temperatures above 32°C and 8 days with maximum values above 38°C. The record-breaking hot year 2024 began with unfavourable values already at the beginning of June, with a significantly higher number of

days with maximum temperatures above 32°C (81), and (4) days with values above 38°C.

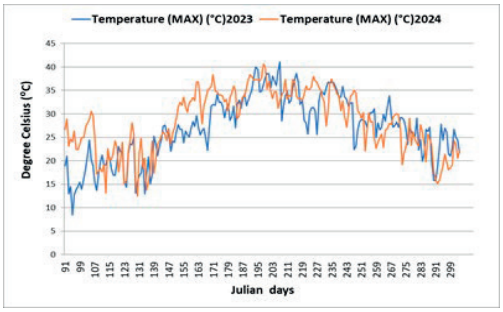


Figure 4. Maximum air temperature during the critical periods (June and July) 2023 and 2024

High RH% can alleviate the impacts of heat on maize seed set by maintaining high pollen viability and a high silk emergence ratio (Dong et al., 2024). Although irrigation reduced stress during the reproductive period, in 2024, 12 consecutive days with average relative humidity between 40% and 50% were observed in July (Figure 5).

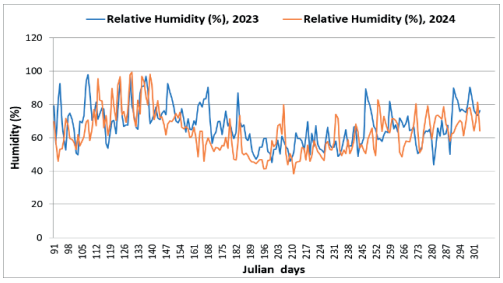


Figure 5. Average relative air humidity in % during the growing season in 2023 and 2024

However, the drip irrigation system significantly improved hydrothermal conditions and reduced abiotic stress in the experimental plots. Two periods are normally defined in the development of corn - the first of which with vegetative manifestations, and the second - the reproductive phases of the species. Corn is more resistant to extreme hydrothermal conditions during the vegetative stages of development, when growth and formation of the optimal leaf area occur. Early sowing is usually a prerequisite for shifting the critical phases forward to less stressful hydrothermal factors. However, there are studies both in favour of earlier (Tomorga et al. 1985; Imholte and Carter 1987) and in favour of later sowing (Herbek et al., 1986). In this case, meteorological data show that the most stressful conditions occurred very early - already in June. The reproductive stage of maize plants between tasseling and silking is the most sensitive stage to temperature fluctuations, as high temperatures accelerate the tassel life cycle (Siebers et al., 2017). Some studies have shown that corn yield decreases linearly with each degree of temperature increase above 30°C (Lobell et al., 2011), and Zhao et al. (2017) reported an estimated 7.4% loss in corn yield with each degree of temperature increase.

The pollen germination starts to decline at 32°C (Herrero and Johnson, 1980; Schoper et al., 1987) and is sharply reduced when the temperature increases up to 38°C (Carberry et al., 1989; Wang et al., 2021). Maize female reproductive organs are wrapped in the husk leaves, which regulate the temperature inside the ear to about 2-3°C lower than the ambient temperature (Khabba et al., 2001). However, temperatures above 38°C can delay the silk emergence time and reduce the silk emergence ratio, which greatly reduces the seed set (Liu et al., 2022). Results from both field and greenhouse experiments have revealed that heat stress during flowering significantly reduces silk receptivity and the silk emergence ratio (Cicchino et al., 2010; Alam et al., 2017; Wang et al., 2021). The data collected during the two experimental years show that the development and especially the reproductive phases of maize plant development occur after conditions of temperature stress. The second year, 2024, is saturated with more extreme events and suggests lower yields in maize plants. However, we believe that late sowing has shifted the critical phases after the most extreme values and in this case has a rather positive effect. Therefore, yields are good, and the chlorophyll index does not reflect significant stress. Another important conclusion is that in the dry summer conditions of Plovdiv, sweet corn cultivation is not possible without irrigation. However, the role of drip irrigation is well demonstrated by improving moisture conditions in both years studied. Negative deviations in the monthly air temperature values were observed only in May and during

both experimental years. The days with stress factors covered almost the entire period of the critical phases of maize, which poses a problem for pollination. The sowing date determines whether the critical phenophase of tasseling and flowering coincides with the most stressful factors, which in the region occur in July. Hybrids with a shorter growing season exhibit better growth parameters when sown later under irrigation conditions.The averaged two-year results for the morphological indicators recorded during the milk maturity phase are presented in Table 1. The control variety Zeaton F1 has the tallest stem, with a significant difference from all other varieties. The varieties HMX5389 F1 and HMX59YS832 F1 showed a significant difference from the shortest variety Turbo F1. The highest leaf number is recorded in the HMX59YS832 F1 variety and the control variety Zeaton F1, which also has the tallest stems. Additionally, HMX59YS832 F1 exhibits a significant difference compared to Zeaton F1. Meanwhile, the Turbo F1 and HMX5389 F1 varieties have a statistically significantly lower number of leaves compared to the control.

The parameters of leaf length and width have close values, with the length naturally varying more than the width. The Zeaton F1, HMX5389 F1, and HMX59YS832 F1 varieties have the longest leaves and belong to the same group. The variety Turbo F1 has the shortest leaves, showing a significant difference from the others. The varieties with the longest leaves also have the widest leaves, but unlike length, the width values are very close, and no significant difference is observed.

The parameter for ear placement height varies from 53.44 cm in the Zeaton F1 variety to 35.33 cm in the HMX59YS832 F1 variety, with a significant difference only between these two. The results for this parameter follow a similar ranking to plant height. For the mentioned indicators, we can conclude that the presence of significant differences indicated that sufficient genetic variation among the hybrid genotypes was present. Similar relationships are reported by Ilker, 2011 in a study with other corn hybrids.

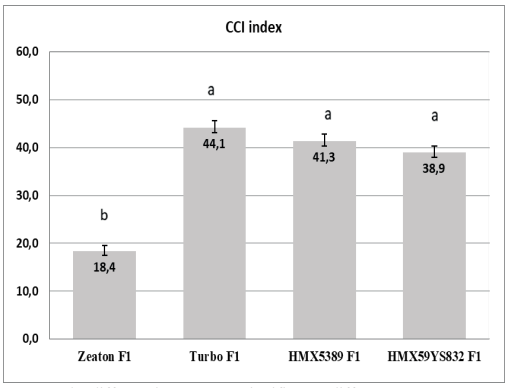
Regarding the number of tassel branches, the variation in results is minimal, and the average values do not show statistical significance.

The recorded ear morphometry does not indicate a significant advantage for any particular variety, either in length or width. The ear length ranges from 25.0 cm in the HMX59YS832 F1 variety to 23.6 cm in the Turbo F1 variety, while the highest ear width is observed in the control variety Zeaton F1.

Figure 6 presents the measured average CCI values from two years of field measurements taken during the tasseling and grain-filling (milk maturity) phases. No structural changes in the leaves due to abiotic or biotic stress were detected during the measurements, indicating that the recorded CCI reflects the normal potential of each variety. The data processing provides a clear and statistically significant separation of the varieties into two groups. The first group consists of three varieties Turbo F1, HMX5389 F1 and HMX59YS832 F1, which have a chlorophyll content index above 38 units. The control variety Zeaton F1 is distinctly separated from this group, with a chlorophyll index below 20 units, and this trend remains consistent between the two measurements. Similar ratios have been observed in field trials for other maize genotypes in previous studies (Dwi Purbajanti et al., 2024; Frommer et al., 2019).

Additionally, all varieties show a growth increase in the chlorophyll content index within the range of 20-25% over the same period. Regarding the relationship between the chlorophyll index and most morphological indicators, no strong correlations were found. However, a stronger negative relationship was identified between the parameters of stem height, ear placement height, and the relative chlorophyll index of the leaves.

Table 2 presents the calculated correlation dependencies between the recorded biometric parameters. The highest correlation coefficients are observed in the interactions between plant height the parameters of leaf length and the number of leaves per plant. The relationship between the number of leaves per plant and leaf length is moderately positive. All mentioned correlation interactions so far are statistically significant at the 1% and 5% levels. These relationships indicate that taller plants lead to a greater number and larger size of leaves.



The different letters mean significance difference at 5%

Figure 6. Average results of the leaf chlorophyll content index during periods 2023 and 2024

Moderately positive correlation coefficients, though not statistically significant, are observed in the relationships between the number of leaves and leaf width, the number of leaves and cob diameter, as well as leaf length with the indicators of cob setting height and cob length. The positive interactions with unproven statistical significance between a greater number and larger leaves and longer ear lengths are expected as a result of improved photosynthetic activity.

On the other hand, the strongest and most significant negative correlation is between leaf length and ear diameter. Moderate negative correlations, though not statistically significant, are observed between the number of leaves and cob diameter, plant height and cob diameter, and the number of leaves and the number of tassel branches. Therefore, in terms of ear diameter, a larger leaf mass not only does not lead to a larger diameter but actually reduces it. Zaluski et al. (2021) reported stronger positive interactions between plant height and the morphometric indicators of the ear, while in our case, the relationships are weakly positive or negative.

The behaviour of four sweet corn varieties under hydrothermal stress conditions was studied. During the first and second experimental years, there were 54 and 81 days, respectively, with maximum temperatures exceeding 32°C. Therefore, the vegetation occurs faster and with shortened interphase periods. Plants were most resilient during their vegetative stages.

Among the four tested hybrids, the control Zeaton F1 demonstrated the highest plant height, leaf width, ear height, and cob width.

The shortest hybrid variety, Turbo F1, exhibited higher morphometric parameters of the ear and the highest chlorophyll content index in the leaves compared to the control.

Experimental data confirm that temperature anomalies and stress factors during critical growth phases can affect pollination and yield. In this case, late sowing mitigated the adverse effects of extreme climatic conditions, contributing to stable yields despite unfavourable hydrothermal conditions. It has been proven that irrigation combined with proper fertilization is essential for cultivating sweet corn in Plovdiv's dry summer conditions. The drip irrigation system effectively improves soil moisture levels, preventing drought stress in the crop.

Table 1. Average results of the biometric parameters -milk maturity phase

Variety/Parameter	Plant high (cm)	Number leaf per plant	Leaf length (cm)	Leaf width (cm)	Height of ear placement (cm)	Number of branches per tassel	Ear length (cm)	Ear width (cm)
Zeaton F1-(control)	153.11	9.44	81.33	11.50	53.44	15.89	24.00	3.06
Turbo F1	115.44 ***	7.44 **	73.00 **	10.67 n.s.	43.78 n.s	16.78 n.s.	23.56 n.s.	3.02 n.s.
HMX5389 F1	136.67 **	8.22 *	80.89 n.s.	10.22 **	49.56 n.s.	15.56 n.s.	24.11 n.s.	2.87 n.s.
HMX59YS832 F1	138.22 *	10.89 *	81.44 n.s.	11.11 n.s.	35.33 ***	15.00 n.s.	25.00 n.s.	2.88 n.s.
GD 5% *	12.08	1.18	4.93	0.95	10.14	2.75	1.69	0.28
GD 1% **	16.17	1.57	6.60	1.27	13.57	3.69	2.26	0.37
GD 0.1% ***	21.23	2.07	8.67	1.67	17.81	4.84	2.97	0.48

n.s. no significant difference

Table 2. Correlation coefficients between biometric parameters in corn hybrids varieties

Parameter	Plant height (cm)	Number leaf per plant	Leaf length (cm)	Leaf width (cm)	Height of ear placement	Number of branches per tassel	Ear length (cm)	Ear width (cm)
Plant height	1	0.55*	0.84**	0.25	0.46	-0.30	0.21	-0.35
Number leaf per plant		1.00	0.55*	0.47	-0.32	-0.17	0.45	-0.45
Leaf length (cm)			1.00	-0.03	0.42	-0.34	0.40	-0.54*
Leaf width (cm)				1.00	-0.36	0.22	0.06	0.13
Height of ear placement					1.00	0.06	-0.15	-0.04
Number of branches per tassel						1.00	-0.27	0.03
Ear length (cm)							1.00	-0.20
Ear width (cm)								1.00

*-correlation is significant at the 5%; **- correlation is significant at the 1%

CONCLUSIONS

Hybrids with shorter growing cycles showed increased growth productivity under irrigation and later sowing conditions. A stronger negative correlation was observed between stem height, ear height, and the relative chlorophyll index of the leaves. The observed genetic variation among the studied hybrids suggests potential for further selection and breeding programs. Strong correlations between plant height, number of leaves, and leaf length indicate that taller plants tend to develop a greater number and size of leaves. This study provides valuable insights for optimizing sweet corn cultivation strategies in regions facing similar climatic challenges.

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MEDICINALS AND CHEMICAL PROPERTIES OF SOME SPECIES OF THE CUCURBITACEAE FAMILY: A REVIEW

Claudia-Sorina LUNGU[1], Costel VÎNĂTORU[2], Bianca MUŞAT[2], Camelia BRATU[2], Florin BURLAN[2], Viorica LAGUNOVSCHI-LUCHIAN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Plant Genetic Resources Bank - for Vegetables, Floricultural, Aromatic and Medicinals Plants, 56 Nicolae Bălcescu Blvd, Buzău, Romania

Corresponding author email: claudiasorina03@gmail.com

Abstract

The Cucurbitaceae gourd family consists of more than 900 species.The plants of this family consist of several edible fruits used in medicine or in different food recipes. The other parts of the plants (steam, leaves, flowers and seeds) can be used in the treatment of different symptoms such as headache, rhinits, fever, anemia, inflamations or poisoning. Cucurbits are rich in carotenoids, saponins, phytochemicals and vitamins.Momordica charantia can be used in the treatment of diabetes, ulcers or liver diseases.Cucumis sativus seeds can help to lower the cholesterol, while the fruits can reduce swelling and burns.Cucumis metuliferus is a great source of anti-oxidants protecting the organism of the cellular damaging caused by the oxidative stress, reducing inflammations and preventing heart diseases.Different parts of Luffa cylindrica can be used in treatment of snake bites, chronic bronchitis, nasal swelling and used in China for anthelmintic and stomachic purposes.This review evaluates the current literature about medicinals and chemical properties of some plants of the Cucurbitaceae family in addition to their positive effects on human health.

Key words: *Cucurbitaceae, medicinal properties, homeopathy, antioxidants, chemical properties.*

INTRODUCTION

The Cucurbitaceae family, commonly known as the gourd or squash family is a diverse and significant group of plants that includes around 113 genera and over 900 species, making it one of the largest plant families (Dhiman et al., 2012).

Cucurbitaceae are annual or perennial vines plants with fibrous or tuberous roots and angular stems. The leaves are alternate with solitary tendrils and exstipulate. The plants are monoecious or dioecious, with unisexual, paniculate, racemose or subumbellate flowers (Xu Z., Chang L., 2017).

The Cucurbitaceae family is not only important for it's culinary uses, but also for it's medicinal and chemical properties, studied for their therapeutic potential as antidiabetic, anti-inflamatory , antioxidant effects and purgative properties (Rolnik and Olas, 2020).

One of the chemical component present in Cucurbitaceae family is cucurbitacin, a compound categorized into 12 classes ranging from A to T and more than 200 derivates, known for anti-inflamatory and anticancer properties, and saponin, a sour compound used in treatment of ulcers (Li Y. et al., 2023).

Cucumis sativus is widely used as food, eaten raw, pickled or in salads. It's medicinal properties have been recignized in homeopathy, especially in skin conditions like redness or dilated pores, intoxications, digestive issues and urinary disoders.

Cucumis metuliferus, also known as African horned cucumber or kiwano is used in treating diabetes. The fruit is orange with spiky outer skin and a jelly- like interior with edible seeds, containing antioxidants and hydrating content due to it's high water content. *C. metuliferus* is a close relative of cucumbers and melons in which they may have diverged from a closely

ancestor species. Rich in vitamins like, vitamin C, A and potassium, helps strenghtening the immune system and may reduce chronic disease (Helm and Hemleben, 1997).

Momordica charantia has been used in traditional medicine for managing diabetes, due to it's effects to lower blood sugar levels. The plant contain momordicin, which have the same effect as insulin. The Latin name Momordica means "to bite'', referring to the jagged edges of the leaf, which appear as if they have been bitten (Kumar et al., 2010).

Luffa cylindrica, known as sponge gourd or *Egyptian cucumber* is widely cultivated in tropical or subtropical regions. The fruit can be eaten when is immature as main dish or in salads. When matured, the fibers are used as bath sponge for exfoliation. The water of luffa is used in Japan in lotions for skin irritations (Umehara et al., 2018).

1. MEDICINAL PROPERTIES

1.1. ANTIBACTERIAL ACTIVITY

The seed extraction of *Cucumis sativus* showed high inhibition against *S. aureus*, using the Agar well diffusion method, the seeds possessing potential broad spectrum in antimicrobial activity (Heidari H., 2012).

Cucumis sativus shows high antibacterial and antifungal activity against microorganisms like *S. pyogene*, *E. coli* and *P. aureginosa*, therefore cucumber can be used as an alternative for antibiotics (Balogun et al., na).

Extracts from leaves, fruits and seeds of *L. cylindrica* have been studied for their preventive effects against *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*.

The extracts of dried and ash-dry leaves of *Luffa cylindrica* contain bacteriostatic and bactericidal effects against pathogenic bacteria on the skin (Sirisa-Ard et al., 2023).

The extracts from *L. cylindrica* exhibited antimicrobial activity against *Candida albicans* and *Staphylococus aureus*.

1.2. ANTIOXIDANT ACTIVITY

It has been shown through a study from Nigeria the extract of *Cucumis metuliferus* contains volative oils, presenting antioxidant activity with IC50 values, ranging between 537.56-2579.91, the study supporting the use of the *C. metuliferus* in the treatment of diseases (Elisha, 2024).

The combined action of flavonoids, tannins and phenolic acids in *Luffa cylindrica* extracts help to combat the oxidative stress, which arise from an unequal state between the generation of free radicals and the body's capacity to counter act this imbalance may cause damage to the cells, associated with various chronic diseases and cancer (Indurthi & Sarma, 2024).

The University of Malakand, Chakdara, Pakinstan, tested the effects of the microwave against the antioxidants properties of *Momordica charantia*.

The fruits were divided into four portions and then subjected to microwave heating for 5-10 and 15 minutes. One portion of untreated fresh fruit was used as a control (Zeb et al., 2024).

The results showed the microwave heating appears to positively influence the concentration of most of the identified compounds of *Momordica charantia* fruits, excepting for one compound (9-z-lutein) which decreased with prolonged heat exposure.

Aqueous extracts of bitter melon have a considerable hypoglycemic impact and glucosis tolerance.

Ethanolic extracts of cucumber fruit shown hepatoprotective activity against paracetamol induced toxicity in albino rats (Heidari et al., 2012).

1.3. ANTI-DIABETIC

Diabetes mellitus is a global health concern, affecting millions of individuals worldwide. It is characterized by hiperglycemia resulting from insufficient insulin production or insulin resistance (Sethi et al., 2024).

The extract of *C. metuliferus* contains 17 known phytochemical compounds, including ursolic acid. The structure was determinate by IR spectroscopy, mass spectroscopy and 1H NMR. *C. metuliferus* exhibited enzymatic inhibitory activity on key enzymes involved in diabetes (Busuioc, 2023).

The study from Romania demonstrated the activity of hydro-ethanolic extracts of *C. metuliferus* in prevention therapy of diabetes mellitus and compounds identified with a potential use in various disease management.

The fruit ethanol extract of *Cucumis sativus* was used to treat diabetes in rats, the study from Benin-Benin inducating the ethanol extract of the plant can enhance antioxidant defensive

system exposed to the diabetogenic agent, STZ (Osahon et al., 2024).
The smoothie made with cucumber and other fruits (carrots, apples, coconut milk) was effective in lowering blood glucose levels in diabetic rats, making the smoothie suitable for type-2 diabetics (Michael et al., 2024).
Luffa cylindrica leaf and seeds extracts showed results in improving the lipid profile and lower the blood glucose in rats (Abigail L. & Metuaghan E.A., 2019).
The antidiabetic activity of *Luffa cylindrica* seeds protein in rats with diabetes has been studied at the University of Ilorin. Luffa cylindrica seed protein (LSPH) was obtained from LCS protein isolate via pancreatin hydrolysys. The study revealed that LSPH may be explored as potential anti-diabetic agent with little or no safety concern (Arise et al., 2024).
A study by Oguntibeju (2019) proved that charantin-rich extract increase plasma glucose tolerance, suggesting the extract could improve insulin sensivity in patients with diabetes type 2. (Oguntibeju, 2019)
There is no approved cure for diabetes mellitus; therefore, the management and treatment of the disease are the primary courses of action. The treatment of diabetes mellitus is complex and involves a combination of exercises, pharmaco-therapy and nutritional therapy as interventions for successful disease management (Shaikh A.A. et al., 2022).

1.4. ANTI-HIV ACTIVITY

M. charantia extracts inhibit the infection and development of most viruses, including the HIV, the plant root having a moderate anti-HIV activity, while the leaf extract exert immunostimulatory effects against other potential viral diseases or worms infections.
In a hospital in Sri Lanka, the diabetes patients were administrate fruits and leaves of *Momordica charantia* in different forms (cooked, fried, crushed, raw or as juice) with no other plants combined (Niluka et al., 2024). The parts of the plants were dried by sun exposure of shade drying, the majority of the patients consumed fresh plant parts.
The fresh plant parts were consumed by the majority of the patients raw or fried to prevent the loss of bioactive compounds and their biological properties, the study revealing important informations about the antidiabetic plants and their preparation or their form of administration in Sri Lanka.

1.5. ANTI CANCER

The bitter melon have a role in controlling cervical cancer, leukemia and nasopharyngeal carcinoma.
Cucumber peel can aid reducing cramps and potentially contribute to colon health by eliminating toxins, offering potential reassurance in colon cancer (Saira et al., 2024).

1.6. HYPERTENSION ACTIVITY

Cucumis sativus has hypotensive properties, the juice being and effective treatment lowering the blood pressure in pregnant women and cholesterol levels in patients with hypertension (Yanti et al., 2024; Kusrina et al., 2024; Mahruri et al., 2024).
Cucumis sativus can reduce blood pressure and urine protein levels in women childbearing age before hypertension (Kartini & Delano, 2024).
The pulp the *Cucumis sativus* can heal the diabetic wounds. An ointment containing silver nanoparticles was applied on mice to assess it's healing potential. The results demonstrated the wound healed in 15 days, while also exhibiting antibacterial and antioxidant properties (Iqbal et al., 2024).

1.7. TRADITIONAL MEDICINE

The cucurbitacins has been used in traditional Chinese medicine for treating liver diseases, hepatitis and hepatic cancer, inhibiting proli-feration of cancer cells (Li Y. et al., 2023).
A recipe from Burkina Faso is used in the treatment of viral hepatitis B, often associated with *Momordica charantia.*
Momordica charantia, used in the treatment of liver cancer, is associated with a recipe from Burkina Faso, used in the treatment of viral hepatitis B (Zongo et al., 2024). Hepatib tiben is a supplement made from whole plants of *Phyllanthus amarus*, *Cassia nigricans* and leaves of *Balanites aegyptiae.*
The study made in Burkina Faso suggest that aqueous infusions of Hepatib tiben and *M. charantia* could be promising due to their high content of total phenolic compounds.
The Hepatib tiben and *Momordica charantia* can be used combined for the treatment of

advanced chronic viral hepatitis and liver cancer, by traditional health practitioners in Burkina Faso (Traoré K.T. et al) but further studies needs for a better understanding.
The compounds found in L. *cylindrica* peel can reduce pain and swelling, while the water extract can be used to treat sunburnt or reducing the redness.
C. sativus is an ingredient used in lotions or masks to reduce the irritations, treat sunburn and heal blemishies.

1.8. HOMEOPATHIC USES

Some varieties of luffa are used in homeopathic medicine as treatment of seasonal allergic rhinits.
A study in Germany demonstrated the treatment produced a significant decrease in hay fever symptoms, with no significant adverse systemic effects (Weiser M. et al., na).

2.0. CHEMICAL PROPERTIES

Phytochemicals are naturally occurring compounds that are biologically active and highly beneficial on human health. The main phytochemical components present in medicinals plants are flavonoids, terpinoids, tannins, saponins, cardiac glycosides, alkaloids, phytoesterols and phenolics (Figure 1).
Phytochemicals can produce direct or indirect defensive against anti-inflamatory, antimicrobial, antioxidative and anti proliferative properties (Sitobo et al., 2024)
Tannins have the potential to act as biological antioxidants, proton precipitating agents and metal ion chelators (Okonkwo S., 2009).
The presence of saponins contribute to a higher degree of antibacterial activities.

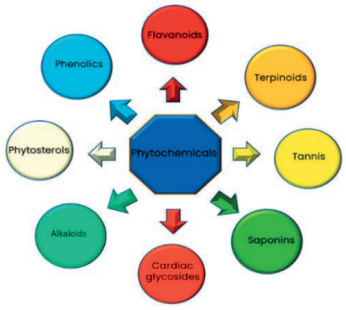


Figure 1.The main phytochemical components present in medicinal plants ((Sitobo et al., 2024; Bansal A. & Priyadarsini C., 2021)

Saponins are found in a variety of plant species through the stems and leaves. The saponins have a sour flavor and is used in the treatment of ulcers or an antibacterial .
The saponins from *Luffa cylindrica* present in the peel and pulp, have desensive role in the plant system against pests and diseases.
Phenols and flavonoids were found in high quantity in *C. sativus* peel, in concentration of 261.73 ± 13.54 for phenols and 261.73 ± 13.54 for flavonoids (Ani et al., 2022).
The phenols and flavonoids play an important role in the prevention of cardiovascular disease and cancer development (Kähköen et al., 1999). These compounds are also important in retaining the oxidative degradation of lipids in food industry, to improve the quality of the food (Wojdyla, 2007).
It has been shown that the ethanol extract of kiwano rind inhibited denaturation of bovine albumin treated with heat in a concentration - dependent manner, exhibiting 47.14% maximum activity (Ani et al., 2022).
The horned melon is more nutritious than cucumber with a higher concentration of vitamins A and C, and zinc. The fruit posses high mineral content particularly potassium, phosphorus, magnesium, calcium, sodium and copper (Muthoni et al., 2024).
Cucumis sativus have higher amount of humidity and protein compared to *C. metuliferus*, while kiwano have higher amount of ash, sugars, polyphenols, flavonoids and tannins (Table 1)
Cucumis metuliferus have less amount of magnesium and calcium compared to *C. sativus* (Table 2).

Table 1.Proximate and phytochemical content of *Cucumis sativus* and *Cucumis metuliferus* (Sambou et al., 2023)

	Cucumis sativus	***Cucumis metuliferus***
Proximate		
Humidity (%)	95.64	93.76
Ash (%)	4.82	7.52
Protein (%)	35.65	27.72
Sugars (%)	430.34	469.99
Phytochemical		
Polyphenols (%)	28.39	49.28
Flavonoids (%)	0.50	0.94
Tannins (%)	22.31	23.30

Table 2. Mineral content of *Cucumis sativus* and *Cucumis metuliferus* (Sambou et al., 2023)

	Cucumis sativus	***Cucumis metuliferus***
Mg (mg/100 g)	43.72	47.87
Ca (mg/100 g)	6.74	21.25
Na (mg/100 g)	19.80	17.02
K (mg/100 g)	214.07	203.90
Fe (mg/100 g)	4.22	1.00

The stem of *Luffa cylindrica* had higher amount of carbohydrate and crude fiber content than the leaf, while the leaf present higher amount of protein and ash than the stem (Sola, 2021).
The proximate and mineral composition of *Luffa cylindrica* leaf and stem are present in the table below (Table 3).

Table 3. The proximate and mineral composition of *Luffa cylindrica* leaf and stem (Sola, 2021)

Parameters	**Leaf**	**Stem**
Moisture	87 ± 0.1	8.17 ± 0.15
Protein	24.47 ± 0.15	11.87 ± 0.15
Ethanol extract	0.73 ± 0.15	0.3 ±0.1
Ash	9.17 ± 0.21	6.77 ± 0.15
Crude fiber	10.57 ± 0.15	18.47 ± 0.15
Carbohydrate	46.37 ± 0.40	54.43 ± 0.42
Ca	180.00 ± 5.00	136.67 ± 7.64
Fe	10.83 ± 0.21	3.70 ± 0.1
Zn	0.4 ± 0.1	0.13 ± 0.06
Mg	73.33 ± 2.89	43.33 ± 2.89
Mn	0.03 ± 0.01	0.02 ± 0.01
K	43.33 ± 2.89	40.00 ± 5.00
Po	14.33 ± 2.89	85.00 ± 5.00

Momordica charantia is a great source of carbohydrates, vitamins and proteins (Table 4), rich in pottasium and B carotene.

CONCLUSIONS

The Cucurbitaceae family have beed used for their antimicrobial, anti-inflammatory and anticancer properties. The cucurbitacins saponins exhibit strong hepatoprotective activities, while the flavonoids and phenolic compounds contribute to their antioxidant and anti-inflammatory effects.
Chemically, these plants are rich in vitamins, minerals and bioactive compound influence the immune functions and help the treating diabetes and different viral diseases. The Cucurbitaceae plants are a great valuable to influence the immune functions and help in treating diabetes and different viral diseases.
The Cucurbitaceae plants are a great valuable source of natural compounds, making them essential in medicinal researches.

Table 4. Proximate and nutrient composition of *Momordica charantia* (Upadhyay, 2015)

Constituents	**Quantity**
Moisture (g/100 g)	83.20
Carbohydrates (g/100 g)	10.60
Proteins (g/100 g)	2.10
Fiber (g/100 g)	1.70
Calcium (mg/100 g)	23.00
Phosphorus (mg/100 g)	38.00
Pottasium (mg/100 g)	171.00
Sodium (mg/100 g)	2.40
Iron (mg/100 g)	2.00
Copper (mg/100 g)	0.19
Manganese(mg/100 g)	0.08
Zinc (mg/100 g)	0.46
B carotene	126.00
Vitamin C	96.00

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DIVERSITY AND RELATIONSHIPS IN KEY TRAITS IN PEPPER COLLECTION OF NATIONAL GENEBANK OF BULGARIA

Veselina MASHEVA, Katya UZUNHALIEVA

Institute of Plant Genetic Resources "K. Malkov", Agricultural Academy, Bulgaria

Corresponding author email: vesi_masheva@yahoo.com

Abstract

To investigate the structure of genetic diversity in pepper germplasm resources in Bulgaria, we compared 52 accessions from GenBank (local varieties and local cultivars). The accessions were characterized based on 14 morphological and vegetative characteristics and ascorbic acid content, fruit dry matter content and sugars. The results of the present study revealed a relatively high level of diversity in the collection. The most significant correlations were found between fruit weight and fruit dimensions - length and width. A positive correlation (>0.5) was reported between vitamin C, dry weight and sugars. A hierarchical cluster analysis was performed and the genotypes were grouped into three clusters. Biplot analysis showed that the genotypes B4E0032, SR and BR from cluster 3 showed a preference for larger values of fresh fruit weight. The significant morphological and biochemical differences between the evaluated specimens are the basis for further breeding programs and a major source of agricultural biodiversity. The evaluation of pepper varieties in the Bulgarian Gene Bank is necessary and important in supporting the development of Capsicum breeding strategies

Key words*: Capsicum annuum, morphology, diversity, characterization.*

INTRODUCTION

Conservation and sustainable use of pepper genetic resources are key to responding to climate change and increasing global food demand in the coming decades. Conservation of genetic diversity is an essential prerequisite for improving plant breeding programs and developing new varieties with desirable agronomic traits and for expanding the genetic base of this economically important crop (Christov, N. et al., 2021). The genetic diversity of germplasm from local varieties is considered one of the criteria for selection of parents for breeding new pepper varieties, due to their competitive advantage (Lanteri and Barcaccia, 2006). Local varieties have higher genetic variability between different germplasm groups, as well as better adaptation to the environment and are an indispensable source of highly co-adapted genotypes. They represent a dynamic and essential component of overall agricultural biodiversity, valued as a source of traits that can be used in scientific breeding programs and to improve the productivity of new crop varieties. Cultivated Capsicum species provide a great diversity in morphological, biological and consumer characteristics of pepper, especially in Central and South America, their geographical origin. As a result of evolution, domestication, and artificial and natural selection in primary and secondary centers of diversity (Garcia B. et al., 2015; Tripodi et al., 2021). Indeed, more than five centuries of pepper breeding in Europe have resulted in a wide variety of local varieties adapted to a wide range of agroclimatic conditions (Garcia et al., 2015).

The Balkans, including Bulgaria, are considered a secondary center of pepper diversity, especially for *C. annuum,* where local forms (local varieties) with diverse phenotypes and qualities have been formed due to specific agroclimatic conditions and growing traditions (Krasteva, L. et al., 2012; Tsonev, S. et al., 2017; Christov, N. et al., 2021). Locally maintained and well-adapted local pepper varieties can still be found in small farms and village yards in Bulgaria and other Balkan countries (Krasteva L. et al., 2009). Characterization of the agro-biodiversity of pepper that exists in Balkan germplasm collections can assist breeding programs by providing more insight into fruit quality. Comprehensive germplasm characterization provides insight into the selection of promising breeding lines that could improve fruit quality and nutritional value of future pepper cultivars. The aim of the study is to assess the genetic diversity of local genotypes of sweet and hot

peppers from the Bulgarian Genebank in terms of morphological, biological chemical characteristics as a basis for future breeding programs and a source of agricultural biodiversity.

MATERIALS AND METHODS

The experimental material included samples of 52 local pepper varieties cultivated in Bulgaria (Table 1), both hot and sweet types.
All experiments were conducted on sandy-clay soil at the Institute of Plant Genetic Resources, Sadovo, Bulgaria.
The experimental design was a randomized complete block with three replications; each plot consisted of 20 plants. Data were collected from 10 plants. Five unharvested plants were left to collect data on full fruit maturity, and five were left to minimize edge effects. Soil preparation, fertilization, and plant protection were carried out according to standard practices for pepper cultivation in Bulgaria.
The data on morphological and physiological traits were determined according to the descriptors of the International Plant Genetic Resources Institute for Capsicum (IPGRI, 1996). The statistical analysis of the data was performed using IBM SPSS Statistics 19.

RESULTS AND DISCUSSIONS

In this study, 52 pepper genotypes from the National Gene Bank of Bulgaria were characterized. As with many plant species, the agronomic and morphological characteristics of pepper genotypes originating from various sources have been studied by numerous researchers (Mutlu and Agan E., 2015; Bozokalfa and Eşiyok, 2010; Cankaya, S. et al., 2010; Virga et al., 2020; Başak, 2019; Taş and Balkaya, 2021; Christov, N. K. et al., 2021; Nankar A.N. et al., 2020).

Descriptive statistics

The mean, maximum, and minimum values for 10 agromorphological traits revealed a wide range of phenotypic variation among the evaluated samples (Table 1).
The analysis results showed that plant height ranged from 45 cm to approximately 60 cm, with most of the samples falling within the 50-58 cm range. The number of branches varied between 3 and 5.

Table 1. Descriptive statistics of the agromor- phological traits

Trait	Min.	Max.	Mean ± SEM	S.D.
Plant Height	32.40	69.0	55.73±0,63	0.94
Embranchment	2.00	6.60	3.64±0,08	0.14
Leave Length	6.78	17.74	11.62±0,22	0.36
Leave Width (cm)	3.20	9.42	6.26±0,13	0.21
Fruit Length (cm)	3.68	19.54	10.60±0,20	0.66
Fruit Width (cm)	1.60	8.20	4.54±0,19	0.29
Wall Thickness (	0.10	0.78	0.36±0,01	0.02
Locules	2.00	5.00	2.68±0,07	0.09
Fruit Weight (g)	7.00	123.40	56.67±2,20	4.98
Flowering Earliness	62.00	89.00	73.08±1,04	1.14
Maturity Earliness	44.00	64.00	49.23±0,80	0.65
Vitamin (%)	13.62	332.98	193.55±2,82	7.99
Total Sugars (%)	2.39	7.82	4.86±0,05	0.20
Fruit Dry Matter (%)	6.61	18.00	10.35±0,60	0.39

Fewer branches indicate a more compact shrub form. The leaf size trait showed significant variation. Specifically, leaf length ranged from 7 to 11.5 cm, while leaf width ranged from 3.3 to 6.5 cm. A larger leaf area suggests more active photosynthetic activity and better overall development. The greatest variation was recorded for fruit weight and vitamin C content The measured traits showed high variability, confirming previous studies (Bozokalfa and Eşiyok, 2010; Başak, 2019).

Correlation analysis

Correlation relationships between the variables are presented in Figure 1.

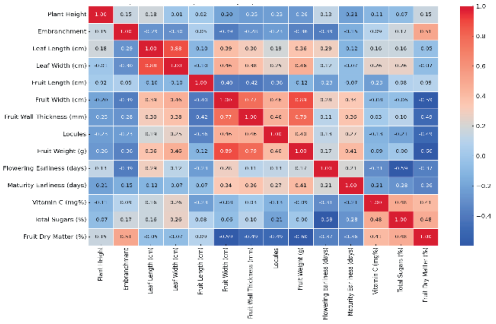


Figure 1. Correlation matrix

Strong positive correlation (~0.7-0.9) was observed between fruit weight and fruit size - specifically, length and width. Our results are consistent with those reported by Matthew et al. (2004) and Ullah et al. (2011) who also found positive correlations for these traits. Positive correlation (>0.5) was also recorded between vitamin C content, dry matter, and sugar content.

Similar results were reported by Naskar et al. (2020).

Moderately positive correlation was found between fruit weight and fruit wall thickness, and a weak positive correlation between number of branches and vitamin C content, as well as between fruit weight and number of locules.

An inverse correlation was observed between earliness and vitamin C content. A negative correlation was also reported by Naskar et al. (2020)

Principal component analysis

PCA was used to identify the differences between the samples as a measure of phenotypic and component variability, and to examine the relative contribution of different biomorphological traits to overall diversity. Principal Component Analysis (PCA) was applied to the studied traits.

According to the results of this study, it was established that traits in the first three principal components (Table 2) should be considered, as they explain a significant portion of the total variation and have loading values greater than 0.3 (Brown, 1991).

Table 2. Eigen vector for Agronomic Characters of the first three Principal Components

	PC1		PC2		PC3
Fruit Width	0.42	Total sugars	-0.49	Leave Length	0.45
Fruit Weight	0.40	Vitamin %	-0.44	Plant Height	0.49
Fruit Wall Thickness	0.38	Leave Width	-0.42	Flowering Earliness	0.42
Fruit dry matter	-0.35	Leave Length	-0.37		
Locules	0.29				

The role of biomorphological characteristics in understanding the divergence between pepper populations was clearly demonstrated in our study and has been previously reported in transcontinental studies of *Capsicum*, with observations made in Brazilian *C. chinense* (Bianchi P.A. et al., 2020), nine Bulgarian accessions (Tsonev S. et al., 2017), the Turkish pepper collection (Bozokalfa et al., 2009), Ugandan hot pepper (Nsabiyera V. et al., 2013), and the Serbian pepper collection (Danojevic D. et al., 2017). Based on PCA analysis and the eigenvectors for the first three principal components, we can make the following summary Principal Component 1 (PC1) explains the largest portion of the variation. Key traits: fruit width (0.42), fruit weight (0.40), fruit wall thickness (0.38), fruit dry matter (-0.35), and locules (0.29). PC1 primarily captures differences related to fruit size and structure. Varieties with larger, heavier fruits and thicker walls have high values in this component. Principal Component 2 (PC2) captures the next level of variation. Key traits: total sugars (-0.49), vitamin C percentage (-0.44), leaf width (-0.42), and leaf length (-0.37). PC2 reflects differences related to leaf morphology and sugar/vitamin content. Varieties with higher sugar and vitamin content and broader leaves score higher on this component. Principal Component 3 (PC3) captures more subtle differences. Key traits: leaf length (0.45), plant height (0.49), and flowering earliness (0.42). PC3 distinguishes varieties by plant stature, leaf length, and phenological traits such as early flowering - i.e., traits associated with vegetative growth and development.

Fruit physical characteristics (size, weight, wall thickness) are most significant in PC1 and explain the greatest variation among the varieties. Sugar and vitamin content, as well as leaf morphology, are critical in PC2. Plant habit and phenology (height and flowering) are weaker contributors but still distinguish varieties in PC3. Figure 2 presents a heatmap comparing two different clustering methods used to group the pepper varieties:: PCA clustering, based on Principal Component Analysis and the K-means algorithm.

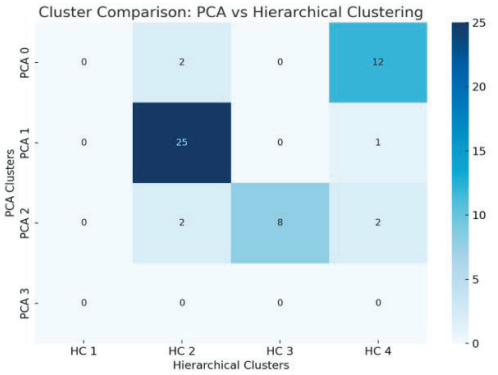


Figure 2. Cluster comparison PCA vc HC

Hierarchical clustering, based on a hierarchical method that creates a dendrogram and stepwise merges the closest groups.

The rows show the groups formed by PCA. The columns show the groups formed by hierarchical analysis. The numbers in the cells indicate the number of varieties assigned to each specific combination of both clustering methods. In our study, most of the varieties (32) appear along the diagonal of the matrix (from the top left to the bottom right), indicating that the two methods yield similar results.

Twelve accessions fall outside the diagonal, meaning they were grouped differently by the two methods.

PCA Biplot: PC1 vs PC2

This biplot combines the information from the PCA analysis: the points represent individual accessions. The red vectors indicate the contribution of each variable to the principal components - the length and direction of each arrow reflect the strength and direction of influence. The biplot analysis of the quantitative traits revealed significant variability among the accessions. The biplot showed that characteristics related to fruit size and structure - such as fruit weight, wall thickness, and fruit width - contributed strongly to PC1while variables such as sugars, vitamin C, and dry matter were more closely associated with PC2. The genotypes PR, BR1, and B0E0341 showed their potential for flowering earliness, maturity earliness, and number of locules.

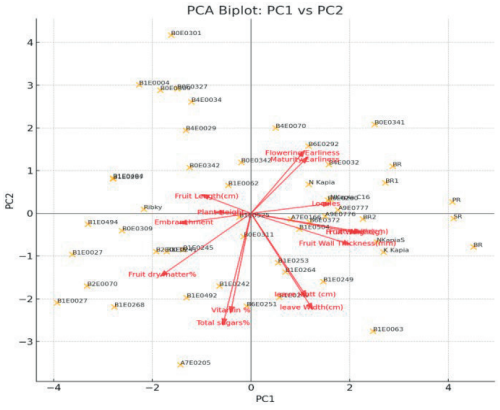


Figure3. PCA Biplot: PC1 vs PC2

SR, BR, and B1E0063 formed a group with larger fruits, thicker walls, and broader and longer leaves.The genotypes were distributed across all four quadrants of the biplot.

Six genotypes - A7E0205, BOE0309, B1E0027, B1E0494, B1E0268, and B2E0070 - formed a separate group on the negative side of PC1 due to their low content of sugars, vitamin C, and dry matter. Four genotypes - BOE0301, B1E0004, B2E0034, and B1E02422 - were grouped on the positive side of PC2 because of their short fruit length.

The remaining 39 genotypes were positioned closer to the center of both axes, as their traits showed similar values.

Cluster analysis

Based on the cluster analysis, the varieties included in this study were grouped into three main clusters (Figure 4), according to the average levels of variation between the accessions generated from the genetic distance matrix.

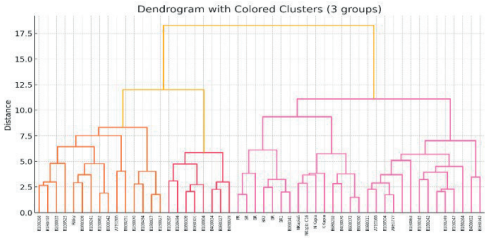


Figure 4. Dendrogram based on 13 quantitative traits of 52 pepper genotypes

Cluster I included 13 accessions, Cluster II had 8, and Cluster III contained the majority-29 accessions. Cluster I: These were medium-height plants with fruits of average weight, but very high vitamin C and sugar content. They have excellent taste - ideal for fresh consumption and as functional foods. They are typically mid-season varieties. Cluster II: These are low to medium-height plants that produce small to medium fruits (longer than they are wide). They have thin walls and low fruit weight. They mature early and are suitable for early production. Cluster III: These are tall plants with larger leaves. The fruits are large, heavy, have thick walls, and contain 3 or more locules. They have high dry matter content. These are usually late varieties, suitable for industrial processing and market-oriented production.

CONCLUSIONS

The results of the present study revealed a relatively high level of diversity within the collection. This information may be useful for initiating a conservation strategy, as well as for supporting an informed breeding and improvement program for pepper.

The most significant correlations were identified between fruit weight and fruit dimensions - length and width - with a strong positive correlation observed. Positive correlation (>0.5) was also noted between vitamin C, dry matter, and sugar content. Hierarchical cluster analysis was conducted, and the genotypes were grouped into three clusters. Principal Component Analysis (PCA) identified three main components.

The biplot analysis showed that genotypes B4E0032, SR, and BR from Cluster 3 showed a preference for higher values in fresh fruit weight.

The results obtained can support the identification of potential genotypes for further breeding or recommendation for direct use in production. The evaluation of pepper varieties in the Bulgarian Gene Bank is necessary and important for developing effective breeding strategies for *Capsicum*.

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GENE EFFECTS INVOLVED IN THE MANIFESTATION OF GROWTH CHARACTERISTICS AND RESISTANCE TO THERMAL STRESS

Nadejda MIHNEA, Galina LUPAȘCU, Vadim RUSU

Moldova State University, Institute of Genetics, Physiology and Plant Protection,
20 Pădurii Street, MD-2002, Chișinău, Republic of Moldova

Corresponding author email: mihneanadea@yahoo.com

Abstract

Data are presented on the response of tomato plant growth characters in hybrid combinations F_1, F_2, BC_1, BC_2 and parental forms to stress temperatures (40, 42, 10°C). The reaction of seedlings to high temperatures (40, 42°C) and low positive (10ºC) was differentiated depending on the combination, character and temperature level. The calculation of the degree of dominance (h_p) of the growth characters (radicle, stem, whole seedling) of tomatoes demonstrated the significant influence of positive (+), negative (-) dominance factors on their phenotype. By researching the genetic effects involved in the phenotype and the inheritance of the radicle and stem length character, it was found that for each combination, in the control (25ºC) and thermal stress (40ºC, 42º and 10ºC) variants, effects with positive values (increasing) and, with negative values (which decrease) the character. The combinations under study showed duplicated epistasis, which reveals the need for long individual selections in order to obtain tomato genotypes resistant to high temperatures.

Key words*: tomatoes, varieties, hybrids, gene effects.*

INTRODUCTION

Temperature is one of the main environmental factors influencing plant growth, development and productivity. Global average temperatures are continuously increasing, and this change has negative effects on crop productivity. Thermal stress (TS) is a major abiotic factor that globally causes fruit decline in many agricultural crops, including tomatoes, thus presenting a problem for food security (Tripathi et al., 2016; Bisbis et al., 2018). The challenges generated by climate change will thus require the implementation of new strategies to adapt the newly created varieties in a timely manner to local conditions to reduce the effect of stress temperatures (Porter et al., 2014; Bisbis et al., 2018).

TS has negative effects on physiological processes in the plant (disruption of photosynthesis, increase in respiration), which leads to decrease in plant productivity (Prasad et al., 2017). Another effect of TS is the negative impact on the root system, which provides support for the plant, nutrient absorption and transport to other plant organs (Valdés-López et al., 2016). These phenomena lead to poor root development, disruption of growth stages, pollination and flowering (Sehgal et al., 2017).

In many species, including tomato, in both cool and hot seasons, productivity decreases as a result of the reduced rate of fruit set. In general, the reproductive period is more sensitive to heat stress than the vegetative period (Mittler & Blumwald, 2010; Ruan et al., 2010; Zinn et al., 2010). Increasing the temperature even by 2-4ºC above the optimal level of 25-30ºC/20ºC - day/night (Camejo et al., 2005), can strongly affect the reproductive organs, especially the viability of the pollen and the development of the gametes (Ayenan et al., 2019; Raja et al., 2019), pollination capacity, causing flower drop and fruit firmness reduction (Firon et al., 2006; Ozores-Hampton et al., 2012). It was found that resistant tomato genotypes have the ability to form a much higher number of fruits than sensitive ones under stress conditions (Comlekcioglu et al., 2010).

The environmental conditions of recent years, presenting a major risk for agricultural crops, require the creation of tomato genotypes with complex resistance to extreme environmental factors (Hazra et al., 2007; Mihnea, 2022).

The quantitative characters of living organisms, including plants, are influenced by several genes, with the participation of additive gene actions and epistatic interactions (Ardiarini et al., 2022), the study of the number of genes and gene actions being necessary for the efficiency of breeding programs (Samak et al., 2011). Characters, for which the proportion of duplicated epistasis with a minus sign is significant, can be improved only in advanced generations, and positive epistatic interactions indicate the existence of complementary gene actions and the need for intensive selection from early to advanced generations (Jayaramachandran et al., 2010).

Higher-order gene interactions frequently influence the genetic bases of complex characters and contribute to their evolution (Dwivedi et al., 2024).The purpose of the research consisted in establishing the influence of high temperatures on some tomato varieties, lines of perspective and the particularities of actions, gene interactions involved in the response reaction of the growth organs at the early ontogenetic stage.

MATERIALS AND METHODS

Dolgonosic, Mary Gratefully, Rufina, Flacara and L 10B, hybrid combinations F_1, F_2, BC_1, BC_2 were used as study material. Varieties and hybrids were tested at 4 temperature levels: optimal - 25°C and thermal stress: 41, 43 and 10°C. The assessment of the resistance of the tomato samples to high and low temperatures was carried out according to the growth capacity of the embryonic radicle, stem and intact seedling for 7 days after maintaining them on day 4 at temperatures of 42° and 43°C within 6 hours, and at the temperature of 10°C - 21 days (Mihnea, 2016). Seedlings kept constant at 25°C served as a control.

The degree of dominance was established based on the formula proposed by Brübeiker (1966):

$h_p = F_1 – 0.5 (P_1+P_2)/ H_p – 0.5 (P_1+P_2)$,

where F_1 - the average value of the character in the F_1 generation;

P_1, P_2 - the average value of the character in the parental forms;

H_p - the average value of the character in the best parental form.

The analysis of gene effects (actions *a - additive*, *d - dominant*, epistatic interactions*: aa, ad, dd*) was performed based on the Gamble model (1962). The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

In the hybrid combinations F_1, F_2, BC1, BC_2, it was found that the reaction of the seedlings to high (40, 42°C) and low positive temperature (10°C) (Figure 1) was differentiated both in the parental forms and in the hybrid combinations and they recorded a inhibition of the radicle length within the limits of 8.1-51.5% in the parental forms at a temperature of 40°C; 13.6-42.9% at 42°C and at 10°C - 87.5-98.9%. A 2.2% stimulation was recorded in the Mary Gratefully variety at the temperature of 42°C. Decreased growth was attested to L 10 B which was 40.9 and 42.9% at the temperature of 40 and 42°C, respectively.

In the case of hybrid combinations, the decrease in radicle length (R.l.) compared to the control varied in the F_1 generation within the limits of 4.8-31.5% at 40°C, 18.1-55.9% - 42°C, 87.5-94.3% - 10°C; F_2 - 5.0-26.7% at 40°C, 11.8-27.2% - 42°C, 84.1-92.5% - 10°C; BC_1 - 12.2-43.6 - 40°C, 23.2-29.5% - 42°C, 88.8-90.0% - 10°C; BC_2 - 14.2-31.3 - 40°C, 2.3-42.2% - 42°C, 81.1-90.9% - 10°C. Mild stimulation (2.2%) was recorded in Mary Gratefully at 42°C. The temperature of 40°C most strongly influenced the hybrids F_1 L 10B x Rufina (31.5%), BC1 (F_1 Dolgonosic x Mary Gratefully) x Dolgonosic (43.6%), BC_2 (F_1 Dolgonosic x Mary Gratefully) x Mary Gratefully (31.3%). A weaker influence at the temperature of 42°C was recorded in F_1 Rufina x Flacara (18.1%), F_2 Dolgonosic x Mary Gratefully (11.8%), BC_2 (F1 L 10B x Rufina) x Rufina (12.3%) and BC_2 (F_1 Rufina x Flacara x Flacara) (2.3%).

In the case of *stem length* (St.l.), a wide range of variability was also recorded in response to stress temperatures. The inhibition of the stem in relation to the control was reported within the limits of 8.4-46.9% at 40°C, 4.9-55.5% - 42°C, 76.3-97.0% - 10°C. Stimulation was attested in 4 cases at 40°C and 3 cases at 42°C. As with the radicle, genotypes were most strongly influenced by 10°C heat stress. Greater than

70.0% inhibition was noted in all genotypes (Figure 1).

Regarding the *length of the seedling* (Sd.l.), its reduction in relation to the control varied between 3.0-51.5% at the temperature 40°C, 2.9-47.0% - 42°C, 80.2-98 .5 - 10°C. At the same time, the temperature of 40°C most strongly influenced the genotypes Flacara (51.5%), L 10 B (43.0%), BC_1 (F_1 Dolgonosic x Mary Gratefully) x Dolgonosic (43.9%), and at temperature of 42°C, inhibition was greater than 40.0% in F_1 Dolgonosic x Mary Gratefully, L 10 B, F_1 L 10B x Rufina, (Figure 1).

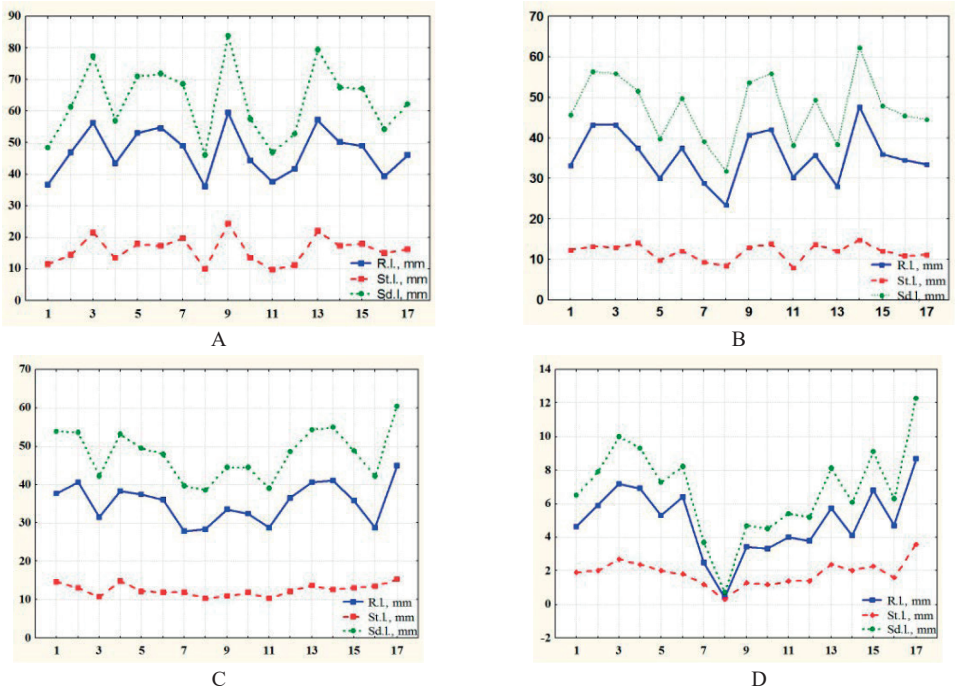


Fig. 1. The influence of optimal temperature:
A - 25°C and thermal stress B - 40°C, C - 42°C, D - 10°C on growth characteristics in tomato

1– Dolgonosic, 2 – Mary Gratefully, 3 – F_1 Dolgonosic x Mary Gratefully, 4 – F_2 Dolgonosic x Mary Gratefully, 5 – BC_1 (F_1 Dolgonosic x Mary Gratefully) x Dolgonosic, 6 – BC_2(F_1 Dolgonosic x Mary Gratefully) x Mary Gratefully, 7 – L 10 B, 8 – Rufina, 9 – F_1L 10B x Rufina, 10 – F_2 L 10B x Rufina, 11 – BC_1(F_1 L 10B x Rufina) x L 10B, 12 – BC_2(F_1 L 10B x Rufina) x Rufina, 13 – Flacara, 14 – F_1Rufina x Flacara, 15 F_2 Rufina x Flacara , 16 – BC_1 (F_1 Rufina x Flacara) x Rufina, 17 – BC_2 (F_1 Rufina x Flacara) x Flacara

Based on the evaluation of tomato genotypes according to three test parameters, it can be concluded that the varieties Dolgonosic, Mary Gratefully, F_2 Dolgonosic x Mary Gratefully, BC_1 (F_1 L 10B x Rufina) x L 10B, BC_2 (F_1 L 10B x Rufina) x Rufina, F_1 Rufina x Flacara, showed complex resistance to high temperatures, and the variety Mary Gratefully and the combination FDolgonosic x Mary Gratefully they are highly resistant, showing interest in tomato breeding.

In order to establish the inheritance pattern of tomato resistance to the mentioned stress temperatures, the degree of dominance was estimated. It was found that according to the level (high/low) and orientation (+/-) the parameter was different and depended on the combination, character and temperature level. In optimal conditions, F_1 hybrids showed positive overdominance, intermediate dominance of radicle, stem and seedling length characters (Table 1).

Stress temperature also influenced the degree of dominance (h_p) of growth characters in terms of degree of expression as well as orientation.

Table 1. Dominance degree of tomato seedling growth characters

The character	F_1 Dolgonosic x Mary Gratefully	F_1 L 10B x Rufina	F_1 Rufina x Flacăra
25ºC			
The length of the radicle	+2.8	+2.64	+0.33
The length of the stem	+6.0	+1.98	+0.23
Seedling length	+3.49	+2.37	+0.29
40ºC			
The length of the radicle	+1.0	+5.2	+9.61
The length of the stem	+0.5	+8.4	+2.61
Seedling length	+0.93	+4.92	+8.03
42ºC			
The length of the radicle	-5.13	+27.5	+1.06
The length of the stem	-3.44	+0.00	+1.00
Seedling length	-116.0	+8.00	+2.16
10ºC			
The length of the radicle	+2.86	+1.82	+0.41
The length of the stem	+8.00	+1.20	+0.64
Seedling length	+4.00	+1.67	+0.46

Notes: $-0.0<h_p<-1$ – negative overdominance; $-1<h_p<-0.5$ – negative dominance; $-0.5<h_p<+0.5$ – intermediate dominance; $+0.5<h_p<+1$ – positive dominance; $+1<h_p<+0.0$ – positive overdominance [Bryubeyker, 1966].

For example, in the control variant h_p for *the radicle length* varied within the limits of +0.33...+2.80, and in the variants with temperatures of 40, 42 and 10°C – within the limits of +1.0...+9.61, -5.13...+27.5 and +0.41...+2.86, i.e. between the overdominance of the parent with the highest radicle growth values to the overdominance to the parent with the lowest values, which denotes the existence of specific interactions of the alleles of the two genomes, function of character, combination and temperature level. In the control variant, the degree of dominance of the *stem length* character varied within +0.23...+6.0. In the variants with temperature 40 and 10°C, the hybrid combinations differed only based on the values of the degree of dominance, which were +0.5...+8.4 and +0.64...+8.0. At the temperature of 42°C, the F_1 Dolgonosic x Mary Gratefully combination recorded negative overdominance (-3.44), the F_1 Rufina x Flacara combination (+1.00) – positive dominance, and the F_1 L 10B x Rufina (h_p=0, 00).

Regarding *seedling length*, in the control variant the degree of dominance varied within the limits of +0.29...+ 3.49, and in the variant with stress temperatures: +0.93...+8.03; -116.0...+8.00 and +0.46...4.00, respectively, at temperatures of 40, 42 and 10°C (Table 1), which once again demonstrates the influence of stress temperatures on the radicle, stem phenotype and tomato seedlings.

By researching the genetic effects involved in the phenotype and the inheritance of the radicle and stem length character, it was found that for each combination, in the control (25°C) and thermal stress (40°C, 42° and 10°C) variants, effects with positive values and negative values were manifested, that increase or decrease, respectively, the character.

Positive additive actions (*a*) with statistical support for which, compared to other effects, are chosen a lot in the selection process, were attested in the control version only for the combination Dolgonosic x Mary Gratefully for the length of the stem (0.61), and for t = 40°C – for the combination Rufina x Flacara for the length of the radicle (1.36) and at 42°C they recorded for the combination – Dolgonosic x Mary Gratefully for both variants.

It should be noted that in the variant with a temperature of 10°C the combination Dolgonosic x Mary Gratefully recorded positive values for the stem length (Table 2).

Epistases *ad*, always contributed to the growth of the radicle and the stem in optimal conditions and to the thermal stress of 40°, 42°C.

Table 2. Gene actions and interactions, involved in the inheritance of tomato growth characters under optimal conditions and thermal stress

	Combination	*m* F_2	*a*	*d*	*aa*	*ad*	*dd*
		25^0C					
		The length of the radicle					
1	Dolgonosic x Mary Gratefully	43,49±1,70*	-1,78±2,14	55,98±1,84*	41,78±1,83*	40,36±2,04*	-60,57±1,99
2	L 10B x Rufina	44,19±1,79*	-4,94±1,80*	-3,51±1,82*	-20,0±1,79*	38,02±1,81*	68,06±1,86
3	Rufina x Flacara	49,04±2,04*	-7,38±2,21*	-23,20±2,09*	-26,52±2,09*	39,44±2,14	50,78±2,14
		The length of the stem					
1	Dolgonosic x Mary Gratefully	13,35±0,80*	0,61±0,72	25,20±0,79*	16,69±0,78	13,57±0,71	-17,90±0,78
2	L 10B x Rufina	13,37±0,83*	-1,65±0,91*	-2,91±0,90*	-12,51±0,85	13,30±0,93	50,51±0,97
3	Rufina x Flacara	18,10±1,46*	-1,60±1,26	-9,69±1,41*	-10,80±1,41	14,52±1,21	15,93±1,34
		40^0C					
		The length of the radicle					
1	Dolgonosic x Mary Gratefully	37,53±1,78*	-7,42±1,55*	-9,70±1,74*	-14,87±1,72	30,85±1,56	42,86±1,69
2	L 10B x Rufina	41,65±1,76*	-5,21±1,96*	-20,48±1,81*	-35,03±1,82	20,88±1,87	36,94±1,87
3	Rufina x Flacara	36,09±1,72*	1,36±1,87	17,07±1,78*	-5,75±1,76	27,22±1,82	16,25±1,85
		The length of the stem					
1	Dolgonosic x Mary Gratefully	14,23±0,70*	-2,28±0,55*	-12,84±0,67*	-12,88±0,66	10,61±0,56	20,45±0,63
2	L 10B x Rufina	13,76±0,66*	-6,0±0,75*	-7,68±0,68*	-11,81±0,68	2,87±0,71	12,33±0,70
3	Rufina x Flacara	12,01±0,58*	-0,12±0,68	2,10±0,64*	-2,86±0,61	10,09±0,70	8,46±0,69
		42^0C					
		The length of the radicle					
1	Dolgonosic x Mary Gratefully	38,30±1,93*	0,55±2,10	-11,10±1,98*	-3,64±1,97	39,54±2,04	-4,84±2,03
2	L 10B x Rufina	32,32±1,78*	-7,77±1,81*	7,20±1,81*	1,88±1,79	20,57±1,81	-9,04±1,84
3	Rufina x Flacara	35,83±1,72*	-15,58±2,04*	11,50±1,83*	5,33±1,81	19,17±2,02	-2,60±1,94
		The length of the stem					
1	Dolgonosic x Mary Gratefully	14,97±0,79*	0,17±0,65	-14,92±0,74*	-11,79±0,75	14,00±0,65	12,79±0,70
2	L 10B x Rufina	11,67±0,66*	-1,78±0,52*	-1,15±0,63*	-1,86±0,63	8,44±0,56	-0,67±0,59
	Rufina x Flacara	13,01±0,64*	-1,70±0,86*	8,24±0,71*	6,35±0,70	10,21±0,83	-13,30±0,78
		10^0C					
		The length of the radicle					
1	Dolgonosic x Mary Gratefully	7,07±0,53*	-1,08±0,39*	-3,17±0,49	-4,71±0,49	4,62±0,40	7,00±0,45
2	L 10B x Rufina	3,22±0,50*	-0,55±0,52	5,33±0,50	3,74±0,50	1,28±0,50	-9,86±0,51
3	Rufina x Flacara	6,97±0,65*	-4,50±0,67*	0,12±0,65	-0,92±0,65	-1,16±0,64	-10,56±0,66
		The length of the stem					
1	Dolgonosic x Mary Gratefully	2,5±0,15*	0,27±0,12*	-1,51±0,14	-2,20±0,14	2,31±0,11	3,89±0,13
2	L 10B x Rufina	1,22±0,11*	0,03±0,10	1,12±0,11	0,67±0,10	1,0±0,10	-1,49±0,10
3	Rufina x Flacara	2,35±0,18*	-2,22±0,35*	2,02±0,25	1,50±0,25	-0,69±0,34	-5,25±0,30

*- p≤0,05.

In the case of the thermal regime 10°C at a single combination – Rufina x Flacara, *ad* effects with negative values were manifested. The most important in character control were the *dominance* effects, the *additive x additive* (*aa*) and *dominant x dominant* (*dd*) epistases.

It should be noted that in most combinations, duplicated epistasis was manifested (opposite orientation of the *d* and *dd* effects), which presents a serious impediment to the improvement process, but also reveals the need for individual, respectively, and long selections in order to obtain tomato genotypes resistant to stress temperatures.

In the Rufina x Flacara combination, complementary epistasis was recorded for radicle length at 40°C, and in Dolgonosic x Mary Gratefully – for stem length at 10°C, which offers chances of success in creating resistant genotypes in limited terms.

CONCLUSIONS

The study of five tomato parents – Dolgonosic, Mary Gratefully, Rufina, Flacara, L 10B, and hybrid combinations F_1, F_2, BC_1, BC_2 in order to see the reaction of growth organs to stress temperatures of 40, 42 and 10°C demonstrated

the differential sensitivity of them which depended on the combination, the growth organ (radicle, stem, whole seedling) and the thermal level.

The degree of dominance of the parents in the F_1 hybrid offspring was of different intensity – from intermediate dominance to overdominance, in most cases being positive.

Different gene actions (*a, d*) and interactions (*aa, ad, dd*) oriented towards increasing (+) or decreasing (-) character were involved in the growth organ phenotype, the most important being *dd* epistases. In most cases, duplicated epistasis was manifested, which creates impediments in the improvement process, however, in the combinations Rufina x Flacara and Dolgonosic x Mary Gratefully, complementary epistasis was recorded in the variants of temperatures 40 and 10°C, which creates premises for selection in narrow terms of resistant genotypes.

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INFLUENCE OF ORGANIC FERTILIZERS ON THE MORPHOLOGICAL DEVELOPMENT AND PRODUCTIVITY IN GREENHOUSE TOMATO

Roksana MINEVA

Trakia University, Agriculture Faculty, Studentski grad Stara Zagora, Stara Zagora, Bulgaria

Corresponding author email: mineva.roksana@gmail.com

Abstract

During the period 2022-2023 was studied, the morphological characteristics were monitored of a greenhouse tomato: plant height, number of leaves, number of inflorescences and number of buds per plant in different variants of treatment with organic fertilizers. The results of the morphological analysis show that in terms of plant height, the variants that were fed with organic fertilizers, on average for the studied period, exceed the unfertilized control by 12.2% to 18.5% for Vermilenza and by 14.3% to 23.6% for Grando. The application of fertilizers increases the development index in the studied tomato varieties. With application of the organic fertilizer Siapton, the highest values were recorded for the Grando variety - 10.50 and for the Vermilenza variety - 7.63. A strong positive relationship was established between the indicators `plant height` and `total yield`, with a correlation coefficient for the Grando variety r = 0.91, and for the Vermilenza variety r = 0.89. The nature of the linear relationship between the indicators `plant height` and `number of inflorescences` was determined, with a coefficient of determination R^2 = 0.846 for the Grando variety and R^2 = 0.797 for the Vermilenza variety.

Key words*: greenhouse tomato, organic fertilizers, productivity, ANOVA.*

INTRODUCTION

Tomato is among the most widely cultivated vegetable crops worldwide (Serio et al., 2006).
It is much appreciated due to its richness in health-promoting compounds such as minerals, carotenoids, vitamins, flavonoids, and polyphenols (Frusciante et al., 2007).
The tomato industry has been searching for new genotypes with improved fruit production, both in the field and industrially processed, together with high-quality performance under sustainable management conditions (De Sio et al., 2021).
Nowadays, using organic fertilizers is an efficient method to achieve sustainable agricultural development. The nutrient release rate of organic fertilizers is slow and hardly exceeds the absorption capacity of plants compared with chemical fertilizers (Guo et al., 2017).
Conventional fertilizers were considered vital for agricultural production to harvest high crop yields. Nevertheless, they are now deemed as environmentally hazardous and an obstacle to sustainable agroecosystems due to intensive chemical inputs that damage the soil over time and have long-lasting impacts (Khan et al., 2024).
The sustainability of agricultural systems is an important global issue. The application of organic fertilizers made from animal excreta or agricultural waste contributes to agricultural sustainability. The physical (structure and water retention capacity), chemical (nutrients and cation exchange capacity) and biological (microflora and microfauna) properties of a soil can be improved by the addition of organic matter (Whalen et al., 2000; Tejada et al., 2003). Long-term studies have shown that the application of organic matter improves crop yield and soil properties including water holding capacity, porosity, and water-stable aggregation and it decreases bulk density and surface crusting of the soil (Edwards et al., 1982; Schjonning et al., 1994).
Organic fertilizers can be of plant, animal or microorganism origin. Among organic fertilizers are composts (e.g., vermicompost, water hyacinth compost, village or town compost), farmyard manure (e.g., cattle and poultry manures), green manures (leguminous and non-leguminous plants) or biofertilizers (algal, fungal and bacterial). The advantages of organic fertilizers include improved physical, chemical, and biological soil properties, enhanced biological activity, slow release of

nutrients, improvement of organic matter, and reduced loss of nutrients. In addition to the benefits related to the environment, organic fertilization has also a positive impact on the quality of vegetable products and harvest quantity (Spencer et al., 2005)

The optimization of the water and fertilization regime in vegetable crops solves a number of problems related to increasing the efficiency of usable irrigation water, the water deficit and a number of environmental problems (Stoyanova et al., 2020). The use of adequate organic fertilization is necessary to promote organic crop production. Organic nutrient sources including compost, manure, compost extract, and authorized fertilizers could be combined and used in order to achieve a balanced nutrient supply and an improved organic tomato yield and quality. (Riahi et al., 2009).

Gao et al. (2023) reported that application of organic fertilizers enhances tomato productivity and fruit quality.

Foliar feeding with organic fertilizers combined with biological fertilization, stimulates vegetative plant activity (Kostadinov et al., 2019)

Organic fertilizers can influence plants growth due to their high nutrient content, high water holding capacity, plant growth regulators, and beneficial microorganisms (Ahmadpour et al., 2020).

MATERIALS AND METHODS

The objects of this study were two indeterminate hybrid greenhouse tomato varieties - Vermilenza F1 and Grando F1 - cultivated in an unheated polyethylene greenhouse near Plovdiv. The study was carried out during the period 2022-2023. The area is flat, with an altitude of 160 m and geographical coordinates N 42° 09', E 24°45' (GPS).

The two tomato varieties were planted under early production conditions, according to the following scheme 110 x 35 (single-row strip) in four replications. Planting was carried out on April 1 and harvested by July 10. The block method with a 10 m² harvest area was applied (Barov, 1982).

The tested varieties are:

VERMILENZA F1 - The fruits are 250-300 g in size. with a deep pink color. Does not crack and does not form a cob. Strong plant with a powerful root system, suitable for long production. Universal variety with excellent transportability. The variety is distinguished by high resistance to: Tomato mosaic virus, Fulvia fulva (Cladosporium fulvum), Verticillium albo-atrum, Verticillium dahliae, Fusarium oxysporum f. sp. lycopersici, Fusarium oxysporum f. sp. radicis-lycopersici. It has medium resistance to: Tomato spotted wilt virus, Tomato yellow leaf curl virus, Oidium neolycopersici, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica.

GRANDO F1 - Large-fruited, indeterminate hybrid tomato, the fruits are flattened and shiny, with a deep red color, without a green ring, hard with a long storage period and resistant to transportation and with very good taste. The hybrid is not demanding on soil type, has a strong root system and high adaptive capacity. Average fruit weight 310-370 g. The hybrid is distinguished by high productivity and resistance to: Tomato mosaic virus, Verticillium albo-atrum, Fusarium oxysporum f.sp. lycopersici, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica

The influence of the basic fertilization carried out with P_{23} (in the form of P_2O_5) and K_{25} (in the form of K_2SO_4) and feeding during vegetation with N_{50} (in the form of NH_4NO_3) and K_{66} (in the form of KNO_3) was studied. Basic fertilization was carried out with 50 kg/da P_2O_5 and 50 kg/da K_2SO_4. During the vegetation until the formation of the fruits, NH_4NO_3 was applied at a dose of 10 kg/da every 10 days. After the formation of the fruits, fertilization was carried out with NH_4NO_3 + K_2SO_4 at 8 kg/da.

The influence of foliar fertilization with amino acid- and carbon-rich fertilizers on the development and productivity of the two tomato varieties was studied, with fertilizers rich in amino acids and carbon. The monitored morphological characteristics included plant height, number of leaves, number of inflorescences, and number of buds per plant. Foliar fertilization during the crop's vegetation was carried out with the products Ergon, Aminototal Grow and Siapton. Three separate treatments were carried out in the 5-6 leaf phase with each of the fertilizers every seven days.

Variants of the experiment:
Variant 1. Control (untreated)
Variant 2. Application of Ergon foliar fertilizer, at a dose of 0.1%
Variant 3. Application of Aminototal Grow foliar fertilizer, at a dose of 0.025%
Variant 4. Application of Siapton foliar fertilizer, at a dose of 0.25%

Characteristics of foliar fertilizers:
Ergon is an organic nitrogen fertilizer that contains 19 amino acids and an extract from algae (*Ascophyllum nodosum*). The content of organic carbon of biological origin (C) is 12%.
Amino Total Grow is an extract of seaweed and contains 18 L-amino acids, organic nitrogen. The content of easily digestible plant L-amino acids in the organic fertilizer is over 43%.
Siapton is a universal organic liquid fertilizer and biostimulator for foliar and soil application for all crops. The specialized bio fertilizer is developed on the basis of natural hydrolyzed proteins. The fertilizer contains organic nitrogen 8.7%, ammonium nitrogen – 0.4%, organic carbon – 25%, total amino acids (of animal origin) – 54.4%, free amino acids – 10%, dry matter – 63%
The soil type is alluvial-meadow soil. It is characterized by a weak humus horizon (average 0.25 m), in which the humus content varies within narrow limits of 1.5 - 2.0%; high water permeability, with low water retention capacity; good aeration and limited field moisture capacity (LFMC) - about 14-16%; with a total porosity of the order of 30 - 42%. (Koinov et al., 1962).
In the soil layer 0-30 cm, the content of nutrient reserves is 117.19 mg/1000 g mineral nitrogen; 6.67 mg/100 g mobile phosphorus and 20.09 mg/100 g available potassium. (Stoyanova et al., 2019).
The irrigation of tomatoes, greenhouse production, was carried out using a drip irrigation system, with built-in drippers at 0.10 cm.
The results were subjected to variance and regression analysis with ANOVA.

RESULTS AND DISCUSSIONS

Tomatoes are an intensive crop, which is distinguished by rapid growth and the formation of a large leaf area. The balance between the vegetative mass and the number of flowers affects the synthesis of plastic substances and, accordingly, productivity. For this reason, morphological and phenological features were studied and monitored in different feeding options. During the two years of the study, morphological features were monitored: plant height, number of leaves, number of inflorescences and number of buds per plant in different treatment options.
During the two years of the experiment, from 30 to 32 irrigations were carried out, depending on the duration of the vegetation period and the requirements of the plants for easily accessible moisture. The irrigation rate of the applied irrigations was 15 m³/da. In the first business year, 32 irrigations were carried out, with an irrigation rate of 480 m³/da. In 2023, thirty irrigations were applied to maintain readily available moisture in the soil horizon, with an irrigation rate of 450 m³/da.

Table 1. Morphological indicators and development of tomato plants at different fertilization levels, Vermilenza

Variant	Stem Height, cm	%	Number of leaves	%	Number of inflorescences	%	Number of tomato clusters	%	Development Index
2022									
1	136	100	12	100	3	100	6	100	
2	152	111,8	14	116,7	4	133,3	7	116,7	5,00
3	153	112,5	13	108,3	6	200	9	150	6,00
4	158	116,2	15	125	6	200	10	166,7	8,00
2023									
1	118	100	15	100	4	100	5	100	
2	135	114,4	17	113,3	5	125	8	160	5,75
3	132	111,9	16	106,7	5	125	7	140	4,50
4	143	121,2	15	100	5	125	8	160	7,25
Average									
1	127	100	13,5	100	3,5	100	5,5	100	
2	143,5	113	15,5	114,82	4,5	128,6	7,5	136,4	5,38
3	142,5	112,2	14,5	107,41	5,5	157,1	8	145,5	5,25
4	150,5	118,5	15	111,11	5,5	157,1	9	163,6	7,63

On average, 31 irrigations were applied during the greenhouse tomato growing season, with an irrigation rate of 465 m³/da. Adams et al. (2019) indicated that the irrigation regime influences the return on investment and the extent of aquifer conservation. The impact of the food and the irrigation system being studied and in terms of their impact on the qualitative composition of tomatoes. They have established relationships between irrigation regimes, nutrient and dry matter content, of soluble sugars, vitamin C and organic acids (Du et al., 2017; Lahoza et al., 2016).
Morphological analyses and phenological observations were carried out from the beginning of reddening of the first fruits at

intervals of seven days (Tables 1 and 2). The conducted studies on stem growth determined the studied varieties as moderately to strongly growing from resumption of growth after planting to tip breakage.

Table 2. Morphological indicators and development of tomato plants at different fertilization levels, Grando

Variant	Stem Height, cm	%	Number of leaves	%	Number of inflorescences	%	Number of tomato clusters	%	Development Index
2022									
1	138	100	13	100	3	100	6	100	
2	156	113	14	107,7	5	166,7	12	200	6,75
3	151	109,4	14	107,7	4	133,3	11	183,3	5,00
4	164	118,8	16	123,1	6	200	13	216,7	9,75
2023									
1	121	100	12	100	4	100	5	100	
2	148	122,3	16	133,3	5	125	7	140	8,50
3	145	119,8	17	141,7	5	125	6	120	7,75
4	156	128,9	18	150	6	150	7	140	11,25
Average									
1	129,5	100	12,5	100	3,5	100	5,5	100	
2	152	117,4	15	120	5	142,9	9,5	172,7	7,63
3	148	114,3	15,5	124	4,5	128,6	8,5	154,5	6,38
4	160	123,6	17	136	6	171,4	10	181,8	10,50

The results of the morphological analysis are presented on average for one plant and show that in terms of plant height, the variants that were nourished with organic fertilizers, on average for the study period, exceed the unfertilized control by 12.2% to 18.5% for Vermilenza and by 14.3% to 23.6% for Grando. The highest values are for the variants fertilized with Siapton. The same trend was also recorded in the analysis of the number of leaves per plant. The results measured for variant 4 are 11.1% and 36.0% higher than the control variant. The results for the number of tillers indicate that the Vermilenza variety forms a 57.1% higher number in variants 3 and 4, while for the Grando variety, the highest values (71.4%) were again established in variant 4. An important indicator of plant productivity is the number of tillers. A positive trend was recorded for the nourished variants for both varieties. Variant 4 of the Grando variety stands out with the highest number of buds, with 81.8% compared to the control, followed by variant 2, with 72.7%. An increase in buds was also found in Vermilenza, but within narrower limits. The indicator is 63.6% higher in variant 4, compared to the control, followed by variant 3, with 45.5%.

A study by Zeleke et al. (2024) found that the synergy between the organic and inorganic fertilizers promoted overall plant health, water efficiency, and productivity.

Organic fertilization makes use of natural resources to enhance soil fertility and therefore is of utmost importance for sustainable agriculture with limited environmental outcomes (Amadou et al., 2020)

The monitored morphological indicators also allow the calculation of a plant development index. In this case, the development index was calculated relative to the control variant. The results show that the application of organic fertilizers increases the development index in both studied varieties. Higher index values were registered in the red tomato, Grando variety, on average for the study period. After applying the organic fertilizer Siapton, a development index ranging from 9.75 to 11.25 or 10.50 on average for the period was recorded. The variation in the Vermilenza variety is from 8.0 to 7.25 in both years and an average for the period of 7.63.

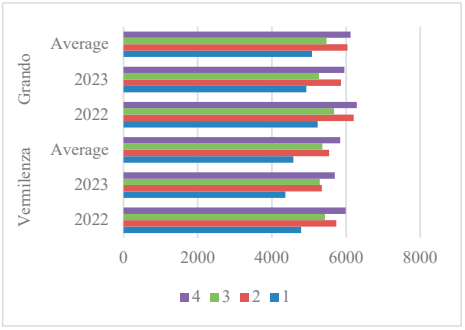


Figure 1. Total tomato yield, greenhouse production, distributed by varieties and variants, kg/da

Figure 1 presents the total yield of the two varieties by variants and years. The results show that when growing tomatoes without applying foliar treatment, the lowest values were recorded for both varieties. The main fertilization of the plants for both varieties provided yields of 5082.0 kg/da for the Grando variety and 4577.0 kg/da for the Vermilenza variety. On average for the study period, the variant treated with the organic fertilizer Siapton (6120.5 kg/da) stood out with the highest productivity for the Grando variety. Productivity was 20.4% higher compared to the control. The same trend was observed for the Vermilenza variety. When applying Siapton, results were recorded that were 27.7% higher compared to the control.

After feeding with Ergon, an increase in productivity was found by 18.8% (Grando) and 21.1% (Vermilenza) compared to the control. Treating plants with Aminototal Grow provides an increase of 7.6% to 17.2%, respectively, in the Grando and Vermilenza varieties.

Combination of organic and inorganic fertilizer treated plots produced higher yield than plots without combination of organic and inorganic fertilizer (Laily et al., 2021).

The combined application of 0.69 g of nitrogen and 75 g of Azolla per pot demonstrated the highest improvements in several key parameters, such as chlorophyll content, photosynthetic rate, stomatal conductance, relative water content, water use efficiency, and plant height. The integration of these fertilizers improves photosynthesis, water efficiency, plant height, and vegetative growth, making it a promising strategy for increasing tomato productivity in sustainable agriculture (Zeleke et al., 2024).

The influence of foliar feeding on the economic early maturity of plants was observed, and it was found that the lowest economic early maturity was observed when feeding with the universal organic liquid fertilizer Siapton (variant 4). In the control variant, the economic early maturity was characterized by the highest values (Figure 2 and Figure 3).

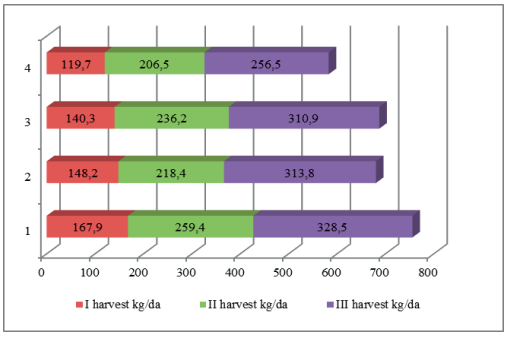


Figure 2. Economic early maturity of tomatoes, greenhouse production, variety Vermilenza, average for the period 2022-2023

The analysis of the results for early maturity of the two tomato varieties shows that the share of the third harvest is the largest, on average for the study period. The highest economic early maturity is observed in the control variant, in which there is only basic fertilization. In all other variants, the economic early maturity is lower than that in the control.

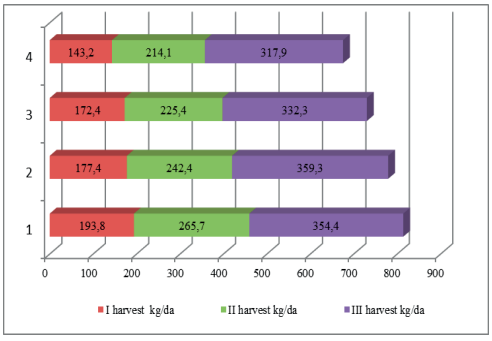


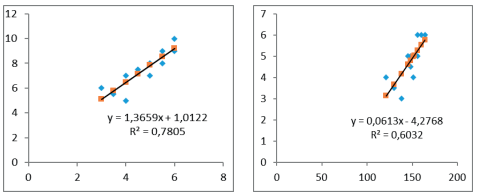
Figure 3. Economic early maturity of tomatoes, greenhouse production, variety Grando, average for the period 2022-2023

Variant 4 stands out with the lowest degree of early maturity, in the Vermilenza variety, respectively with a yield of economic early maturity of 582.7 kg/da. Productivity is 22.9% lower than in the control. While in the other variants, early maturity varies from 9.05% to 9.98%, in variants 2 and 3, respectively. A similar trend was also observed in the Grando variety, but the decrease in early maturity is 17.04% in variant 4. While in variant 2 it is only 4.28% lower compared to the non-fertilized variant. Against the background of the same irrigation regime and basic fertilization, a decrease in early maturity is reported with the application of organic fertilizers during the crop's vegetation. An analysis of variance was conducted on the results for economic early maturity, which has a significance level of $P<0.01$.

Linear regression equations were developed to determine the relationship between the studied indicators. Linear regression is a statistical method for establishing a relationship between the independent variable (height) and the dependent variable (number of inflorescences).

The correlation coefficient was calculated, which determines the strength of the relationship between the indicators. A strong positive relationship was established between the indicators "plant height" and "total yield", with a correlation coefficient for the Grando variety $r = 0.91$, and for the Vermilenza variety $r = 0.89$. High values are also observed in the correlation coefficients between the traits

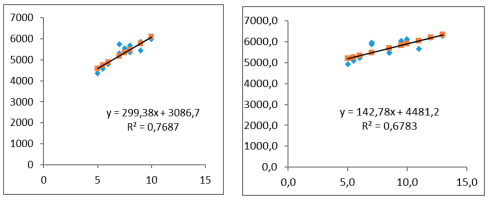
"plant height" and "number of inflorescences", respectively r = 0.88 (Vermilenza) and r = 0.78 (Grando). A high degree of positive correlation is also registered between "total yield" and "number of bunches per plant", with correlation coefficients r = 0.87 for the Vermilenza variety and r = 0.82 for the Grando variety.



a) Vermilenza b) Grando

Figure 4. Linear regression model between number of inflorescences and height in Vermilenza and Grando

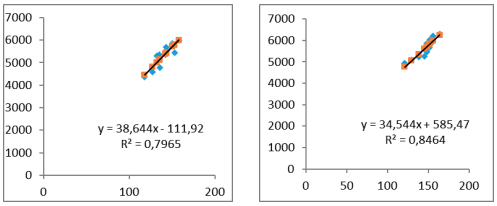
The coefficients of determination for Vermilenza (R^2 = 0.7805) and Grando (R^2 = 0.6032) were calculated. They show that 78.05% and 60.32% of the number of buds, respectively, depend on the height of the plants.



a) Vermilenza b) Grando

Figure 5. Linear regression model between the indicators Number of tomato bunches and yield

Linear regression analysis was used to present the nature of the relationship between the studied indicators and yield. The coefficient of determination (R^2 = 0.7687) was calculated for the Vermilenza variety and R2 = 0.6783 for the Grando variety.



a) Vermilenza b) Grando

Figure 6. Linear regression model between the indicators "height of a plant" and "number of inflorescences"

The analysis shows that when studying the relationship between the indicators "plant height" and "number of inflorescences", the coefficient of determination is high in both varieties, respectively in Vermilenza - R^2 = 0.7965 and in the Grando variety - R^2 = 0.8464. The nature of the relationship shows that 79.7% and 84.6% of the number of inflorescences depend on the height of the plants in the Vermilenza and Grando varieties.

Regression relationships were determined between fertilization with the macronutrients nitrogen, phosphorus, and potassium on the growth and productivity of tomato seedlings, and a high degree of influence of nitrogen on the leaf area index was found, while excessive use contributed to the deterioration of tomato quality (Patanè et al., 2010).

CONCLUSIONS

As a result of the analyses, some main conclusions were drawn:

The application of organic fertilizers increases the development index of the studied tomato varieties. On average, for the period after the application of the organic fertilizer Siapton, the highest index values were recorded for the Grando variety - 10.50 and for the Vermilenza variety - 7.63.

Against the background of the same irrigation regime and basic fertilization, a decrease in early maturity was recorded with the application of organic fertilizers during the growing season of the crop. The lowest economic early maturity was recorded in the Vermilenza variety, being 22.9% lower compared to the control.

A strong positive relationship was established between the indicators "plant height" and "total yield", with a correlation coefficient for the Grando variety r = 0.91, and for the Vermilenza variety r = 0.89.

The nature of the linear relationship between the indicators "plant height" and "number of inflorescences" was determined, with a coefficient of determination R^2 = 0.846 for the Grando variety and R^2 = 0.797 for the Vermilenza variety.

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RESPONSE OF CATALASE TO MICROBIOLOGICAL AND CHEMICAL SOIL INDICATORS IN SOLE AND INTERCROPPED CABBAGE CULTIVATION

Gergana MLADENOVA[1], Boyka MALCHEVA[2], Milena YORDANOVA[1]

[1]University of Forestry, Faculty of Agronomy, 10 Kliment Ohridski Blvd, 1797, Sofia, Bulgaria
[2]University of Forestry, Faculty of Forestry, 10 Kliment Ohridski Blvd, 1797, Sofia, Bulgaria

Corresponding author email: g.mladenova@ltu.bg

Abstract

The influence of microbiological and chemical soil indicators on catalase activity in soils under sole and intercropped cabbage cultivation with leek, beans, tagetes, flower mix, and dill has been established. The highest catalase was observed in intercropping cabbage with tagetes (1.63 ml 0_2/30 min.), while the lowest was found in cabbage and leek (1.20 ml 0_2/30 min.). A high correlation was found between catalase and the total microflora in the soil (r=0.90), mainly determined by the activity of non-spore-forming bacteria (r=0.71) and mold fungi (r=0.82). A moderate correlation exists between mineralization activity and catalase (r=0.39), as well as between total microflora and mineralization coefficient (r=0.36). The relationship between catalase and ammonium (r=0.53) and potassium ions (r=0.33) is moderate, while no such relationship was found with phosphorus (r=0.02). The dependence between Fe and catalase is also moderate. A weak influence on catalase activity in the variants was observed from the quantity of bacilli (r=0.30) and actinomycetes (r=0.27), as well as from the content of humus (r=0.18) and carbon (r=0.17). There is no positive correlation between pH and catalase (r=-0.24).

Key words*: catalase, soil microflora, agrochemistry, co-planting, correlations.*

INTRODUCTION

Catalase is a respiratory enzyme, a sensitive biochemical marker indicative of the activity of soil microorganisms. Many soil microorganisms produce the enzyme catalase - bacteria, actinomycetes, micromycetes.

In agrogenic soils, many authors have established a stronger development of bacteria (especially non-spore-forming) and a weaker development of mycelial microorganisms (actinomycetes and molds), mainly aerobic and mesophilic microorganisms (Naskova et al., 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Frene et al., 2022; Guo et al., 2022; Mladenova et al., 2023; Mladenova et al., 2024; Koleva et al., 2024).

These groups of microorganisms produce the enzyme catalase, which decomposes hydrogen peroxide to water and oxygen, releasing oxygen and an additional water molecule for Fe (heme) catalases (Chabot et al., 2020). Microbiological, mineralization and catalase activity depend on a complex of factors: amount and composition of soil microflora, soil temperature and moisture, sampling depth, content of organic matter and nutrients in the soil, soil type, mechanical composition, soil pH, type of vegetation, research methods, applied agrotechnical measures, the presence of inhibitors and other factors (Uzun and Uyanöz, 2011; Naskova et al., 2015; 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021).

In addition to microbial origin, there is also catalase of plant origin (Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021), higher in the co-cultivation of vegetables (Mladenova et al., 2023, 2024), as well as in broadleaf vegetation compared to coniferous vegetation (Malcheva et al., 2023).

Combined cultivation increases enzyme activities, including catalase activity, and the effect depends on the category of the enzyme, the type of plant, and other factors (Curtright and Tiemann, 2021; Mladenova et al., 2023).

Catalase contains iron in its non-protein component and some authors indicate a dependence of catalase on the iron content in the soil, as well as on the humidity and organic

matter content in the soils (Grozeva and Nustorova, 1995; Mladenova et al., 2024).

Some authors have found a positive correlation between catalase activity and soil potassium and phosphorus levels, but the relationship with nitrogen is statistically insignificant (Türkay et al., 2024).

Catalase has a biological origin (organic matter, living and dead inhabitants) and abiotic origin (soil-forming rocks containing iron and manganese) (Grozeva and Nustorova, 1995; Hristov, 2009).

Soil catalase activity is determined as a function of the state of soil aeration, with its activity decreasing under hypoxic conditions (Brzezińska et al., 2005). Catalase is a key biological marker reflecting microbial activity and soil aeration for early diagnosis and assessment of soil health, and guiding sustainable soil management practices (Türkay et al., 2024).

In a comparison of garden soils and permafrost and high-altitude soils, Chabot et al. (2020) found that microbial communities experiencing higher levels of natural oxidative stress had higher basal intracellular catalase concentrations and catalase specific biomass activities. Other studies have shown that agronomic practices such as fertilization affect catalase activity, mainly indirectly, by affecting the development and activity of soil microorganisms (Naskova et al., 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021). While desert soils are dominated by Cyanobacteria and Proteobacteria (Mogul et al., 2017), garden, well-irrigated soils are typically dominated by Actinobacteria, Proteobacteria, and Firmicutes (Kim et al., 2013; Maksimova et al., 2016), with the former having two times lower catalase activity (Chabot et al., 2020).

Soil microbial parameters and enzyme activity are key indicators of soil health and fertility, and changes in land use and management practices affect them (Meena and Rao, 2021). Microbial diversity and catalase activity are key diagnostic indicators of the biological and biochemical state of soils.

The aim of the present study was to analyze the response of catalase to the influence of microbiological and chemical soil parameters in sole and intercropped cabbage cultivation.

MATERIALS AND METHODS

Agrogenic soils were studied, with sole and intercropped cultivation of cabbage, from the area of the Educational and Experimental Field of the Forestry University in the district Vrazhdebna, Sofia. The variants are as follows:

► V0 - control - soil without vegetation, before starting the experiment;

► V1 - sole cabbage cultivation - *Brassica oleraceae* L. var. *capitate* L.;

► V2 - intercropped cabbage cultivation - cabbage and leek - *Brassica oleraceae* L. var. *capitate* L. and *Allium porrum* L.;

► V3 - intercropped cabbage cultivation - cabbage and beans - *Brassica oleraceae* L. var. *capitate* L. and *Phaseolus vulgaris* L.;

► V4 - intercropped cabbage cultivation - cabbage and tagetes *Brassica oleraceae* L. var. *capitate* L. and *Tagetes patula* L.;

► V5 - intercropped cabbage cultivation - cabbage and flower mix (calendula *Calendula officinalis* L., borage *Borago officinalis* L., blue cornflower *Centaurea cyanus* L., phacelia *Phacelia* sp. Jus., buckwheat *Fagopyrum esculentum* Mill.);

► V6 - intercropped cabbage cultivation - cabbage and dill - *Brassica oleraceae* L. var. *capitate* L. and *Anethum graveolens* L.

The samples were taken from the root zone, at a depth of about 25 cm, and for microbiological analyses, the sampling was carried out sterilely. Microbiological analyses were carried out by the method of limiting dilutions and subsequent cultures on solid nutrient media, specific for each of the studied groups of microorganisms- Meat peptone agar for non-spore-forming bacteria and bacilli, Czapek-Dox agar for mold fungi and Actinomycete isolation agar for actinomycetes and bacteria assimilating mineral nitrogen (Gushterov et al., 1977; Mishustin & Emtsev, 1989). The cultures are cultivated in a thermostat at 25 °C. The total microflora (sum of bacteria, actinomycetes and micromycetes) and the mineralization coefficient (bacteria assimilating mineral nitrogen/(non-spore-forming bacteria + bacilli) were determined by calculation (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

Catalase was determined by the titration manganometric method (Khaziev, 1976).

Nitrogen was determined by the Kjeldahl method, phosphorus by the Petko Ivanov method, humus by the Tyurin method, total carbon - by calculation (Donov et al., 1974). Potassium and iron were determined by atomic absorption spectrophotometer. The active reaction was determined potentiometrically in an aqueous extract.
Correlation analysis was used as a statistical method, implemented using the Excel 2010 program.

RESULTS AND DISCUSSIONS

The biogenicity of the studied soils was higher when cabbage was grown together with: beans, tagetes, flower mix and dill (higher than the control) than when cabbage was grown alone and in combination with leeks (lower than the control) (Table 1).

Table 1. Quantity and composition of soil microflora (CFU/g)

Variants	Total micro-flora	Non-spore-for-ming bacteria	Bacilli	Acti-nomycetes	Micro-mycetes	Bacteria assimi-lating mineral nitrogen
V 0	1909940	1085800	76540	694200	53400	4722933
V 1	1260720	778940	406650	53350	21780	2292630
V 2	1093200	447300	561790	73460	10650	1021750
V 3	2328120	982160	1198440	94720	52800	3615520
V 4	2809775	1885230	770650	104775	49120	2160040
V 5	2390740	1686150	628130	50550	25910	1179310
V 6	2618440	1926930	606950	35600	48960	1958040

The total microflora is highest in the combined cultivation of cabbage and tagetes (V4), and lowest in cabbage and leeks (V2). In all variants, the amount of bacteria is higher than that of mycelial forms (actinomycetes and molds). In V1 (cabbage), V4 (cabbage and tagetes) and V5 (cabbage and flower mix) the distribution of microorganisms in the composition of the total microflora follows the descending order: non-spore-forming bacteria > bacilli > actinomycetes > molds. A similar distribution of the main groups of microorganisms in agrogenic soils is also indicated in other studies (Naskova et al., 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Frene et al., 2022; Guo et al., 2022; Mladenova et al., 2023; 2024; Koleva et al., 2024). While in V2 (cabbage and leek) and V3 (cabbage and beans) the amount of bacilli is higher than that of non-spore-forming bacteria, in V6 (cabbage and dill) molds are more than actinomycetes. In the control, before the start of the experiment - V0, the amount of actinomycetes is higher than that of bacilli. The percentage of non-spore-forming bacteria in V4, V5 and V6 is about 70%, in V1 and V0 about 60%, in V2 and V3 about 40%. The participation of bacilli is as follows: about 50% in V2 and V3, about 30% in V1, V4 and V5, about 20% in V6 and only 4% in the control. The bacilli are spore-forming and adapt better to adverse conditions and changes in the environment. The lower percentage of bacilli in V0 is due to a higher percentage of actinomycetes - 36%. In the other variants, the amount of actinomycetes varies from 1 to 7%, and that of micromycetes is 1-2 in the variants with cultures and 3% in the control. The mineralization coefficient (MC) was determined from the ratio of bacteria assimilating mineral nitrogen and the sum of ammonifying bacteria (non-spore-forming bacteria and bacilli) (Figure 1). The results show that mineralization activity does not depend only on the amount of microorganisms.

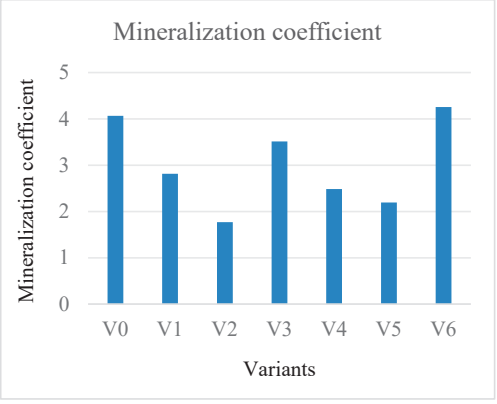


Figure 1. Values of mineralization coefficient

The highest MC values are at V6 (cabbage and dill), followed by V0 (control) and V3 (cabbage and beans), and the lowest rate of organic matter decomposition is at V2 (cabbage and leek). Mineralization activity also depends on a group of factors: composition and quantity of microorganisms, soil humidity and

temperature, humus and nutrient content, vegetation type and other factors (Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Mladenova et al., 2023; 2024; Koleva et al., 2024). The enzymatic activity of soil microorganisms is represented by the values of the enzyme catalase - an exoenzyme that contains iron. The values of catalase and iron are presented in Figure 2.

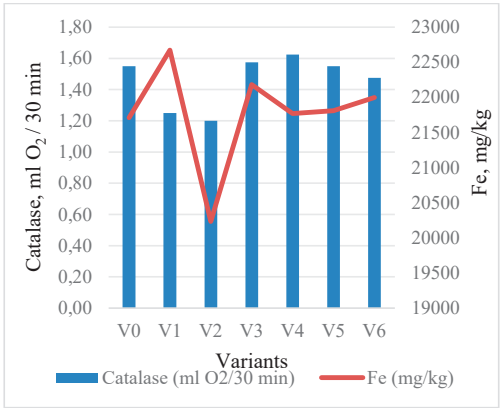


Figure 2. Dependence of catalase on iron

Catalase activity of soil microorganisms is highest in V4 (cabbage and tagetes) and lowest in V2 (cabbage and leek). This trend correlates with iron content to some extent in V4, while in V2 iron has the lowest value. Mineralization activity and enzyme activity are related to the humus and carbon content of the soils, the values of which are presented in Figure 3.

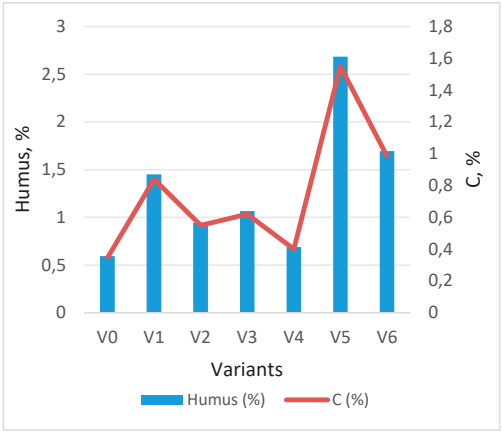


Figure 3. Humus and carbon content (%)

The organic matter content is higher in the variants with agricultural crops compared to the control, without vegetation. The highest values of humus and carbon are in V5 (cabbage and flower mix), followed by V6 (cabbage and dill) and V1 (cabbage), and the lowest in V4 (cabbage and tagetes) and V0. Catalase activity depends on a complex of factors - amount and composition of soil microflora, soil humidity and temperature, iron and nutrient content, type of vegetation, independent and joint cultivation of agricultural crops and other factors (Grozeva and Nustorova, 1995; Uzun and Uyanöz, 2011; Naskova et al., 2015; 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021; Mladenova et al., 2023; 2024). The nutrient content is presented in Table 2.

Table 2. Nutrient content in soils

Variants	N, %	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	P (mg/kg)	P_2O_5 (mg/100 g)	K (mg/kg)	K_2O (mg/100 g)
V0	0.134	36.84	13.18	899.33	43.85	2255	19.17
V1	0.128	12.18	8.50	899.33	51.23	2317	14.90
V2	0.112	18.42	13.88	865.77	45.08	2071	16.00
V3	0.108	14.73	11.90	899.33	43.44	2296	16.60
V4	0.108	24.65	0.00	856.06	43.85	2112	14.90
V5	0.116	34.29	7.93	885.91	49.59	2194	17.90
V6	0.101	21.82	5.67	892.62	50.41	2235	14.80

The studied soils are well-stocked with nutrients. The content of total nitrogen is highest in the control and lowest in the co-cultivation of cabbage and dill (V6). The values of the available forms of nitrogen are highest in V0 and V5 for ammonium ions, V2 and V0 for nitrate ions. Total phosphorus has the highest values in V0, V1 and V3, and the lowest in V4. The share of available phosphorus is highest in V1 and V6, and lowest in V3, V4 and V0. The content of total potassium is highest in the independent cultivation of cabbage (V1) and lowest in the co-cultivation of cabbage and leek (V2). The available potassium has the highest values in the control, followed by the combined cultivation of cabbage and flower mix (V5), and the lowest values in the combined cultivation of cabbage and dill (V6), cabbage and tagetes (V4) and the independent cultivation of cabbage (V1). These discrepancies in the trends for total and

available values are also due to the activity of soil microorganisms. The correlation dependences of catalase on the studied indicators are presented in Table 3.

Table 3. Values of correlation coefficient

Indicator	TM	Bacteria	Bacilli	Act.	Mold	B-N	MC	Cat.	pH	Humus	C	Fe	N	NH_4	NO_3	P	P_2O_5	K
TM	1.00																	
Bacteria	0.91	1.00																
Bacilli	0.42	0.15	1.00															
Act.	-0.08	-0.14	-0.63	1.00														
Mold	0.73	0.53	0.18	0.43	1.00													
B-N	0.11	-0.12	-0.18	0.79	0.72	1.00												
MC	0.36	0.28	-0.14	0.46	0.78	0.71	1.00											
Cat.	0.90	0.71	0.30	0.27	0.82	0.43	0.39	1.00										
pH	0.10	0.13	0.68	-0.99	-0.40	-0.77	-0.49	-0.23	1.00									
Humus	0.11	0.27	-0.40	0.21	-0.07	-0.02	0.16	0.17	-0.28	1.00								
C	0.11	0.27	-0.40	0.21	-0.07	-0.01	0.17	0.17	-0.28	1.00	1.00							
Fe	0.34	0.34	0.11	-0.05	0.43	0.37	0.49	0.34	0.03	0.23	0.23	1.00						
N	-0.53	-0.45	-0.74	0.68	-0.15	0.48	0.05	-0.20	-0.70	0.34	0.34	0.17	1.00					
NH_4	0.35	0.42	-0.49	0.62	0.26	0.21	0.12	0.53	-0.64	0.64	0.64	-0.16	0.32	1.00				
NO_3	-0.68	-0.80	-0.21	0.39	-0.28	0.30	0.05	-0.42	-0.40	0.16	0.16	-0.36	0.43	-0.02	1.00			
P	-0.12	-0.17	-0.16	0.29	0.26	0.55	0.67	0.02	-0.34	0.50	0.50	0.62	0.41	-0.03	0.45	1.00		
P_2O_5	-0.12	0.20	-0.23	-0.46	-0.40	-0.48	0.01	-0.38	0.37	0.52	0.52	0.41	0.01	-0.16	-0.21	0.33	1.00	
K	0.00	-0.07	-0.02	0.18	0.35	0.58	0.64	0.11	-0.21	0.32	0.32	0.84	0.37	-0.19	0.20	0.93	0.33	1
K_2O	-0.04	-0.15	-0.36	0.74	0.16	0.49	0.14	0.33	-0.74	0.59	0.59	-0.15	0.56	0.75	0.57	0.34	-0.34	0.1

Legend: TM - Total microflora; Act. - Actinomycetes; B-N - Bacteria assimilating mineral nitrogen; MC - Mineralization coefficient; Cat. - Catalase.

A high correlation was found between catalase and the total microflora in the soil (r=0.90), mainly determined by the activity of non-spore-forming bacteria (r=0.71) and mold fungi (r=0.82). A moderate correlation exists between total microflora and mineralization coefficient (0.36), and total mineralization activity and catalase (r=0.39). The relationship between catalase and ammonium (r=0.53) and potassium ions (r=0.33) is moderate, while no such relationship was found with phosphorus (r=0.02). Iron is involved in the structure of catalase and the dependence between Fe and the enzyme is also moderate (r=0.342). A weak influence on catalase activity in the variants was observed from the quantity of bacilli (r=0.30) and actinomycetes (r=0.27), as well as from the content of humus (r=0.18) and carbon (r=0.17). There is no positive correlation between pH and catalase (r=-0.24). The pH value in the control was 7.2, and in all other variants - 7.6, i.e. there was no variation in the value in the variants with agricultural crops. Some authors found a positive correlation between catalase activity and the levels of potassium and phosphorus in the soil, but the relationship with nitrogen was statistically insignificant (Türkay et al., 2024). Other studies indicate that catalase activity depends significantly on the humidity of arable soils with vegetable cultivation (Mladenova et al., 2024).

CONCLUSIONS

Catalase activity is very much dependent on the amount and composition of the soil microflora. It is mainly determined by the development of non-spore-forming bacteria and mold fungi, despite the fact that the former have a high percentage participation, and the latter the lowest percentage participation in the composition of the total microflora. The total microflora and catalase activity are highest in the combined cultivation of cabbage and tagetes, and lowest in cabbage and leek.

There is a moderate correlation between iron and catalase. The relationship between catalase

and ammonium and potassium ions is moderate, while such a relationship has not been established for phosphorus. A weak influence on the activity of catalase in the variants is observed by the quantity of bacilli and actinomycetes, as well as the content of humus and carbon. The dependence between the rate of mineralization and catalase is moderate. The mineralization activity does not depend entirely and solely on the quantity of microorganisms (moderate correlation) - the highest values of the mineralization coefficient are in cabbage and dill, and the lowest in cabbage and leek. There is no correlation between catalase and soil pH.

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IMPACT OF COMPOST APPLICATION ON SOIL AND ZUCCHINI YIELD

Gergana MLADENOVA, Boriana PLASHKOVA, Vera PETROVA, Milena YORDANOVA, Tsvetelina NIKOLOVA

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: vera_zamfirova@abv.bg

Abstract

The field experiments were conducted in 2024. The soil type is classified as alluvial-meadow with a light mechanical composition. The crop grown was Cucurbita pepo var. giromontia (zucchini). Four variants of compost treatment were developed, with a tested compost application rate of 5 t/da. The treatments included: Variant 1 (control) - untreated soil; Variant 2-20% of the optimal compost rate; Variant 3-50% of the optimal rate; and Variant 4-100% of the optimal rate. The compost used in the experiment was prepared from urban waste provided by the `Capital Waste Treatment Enterprise.` Compost analysis revealed a nitrogen (N) concentration of 16.2 mg/kg, phosphorus (P) at 2.183 g/kg, and potassium (K) at 7.52 g/kg. Measurements included climate data, biometric parameters, and yield. The results showed that the highest compost application rate (Variant 4) had a depressive effect on plant development, suggesting that excessive compost may hinder growth. The study aimed to evaluate the influence of different compost treatments on the yield and quality of zucchini.

Key words*: compost treatment, zucchini yield, urban area.*

INTRODUCTION

Soil fertility management is a key factor in sustainable agriculture, influencing both crop productivity and environmental health. Among the various soil amendments, compost has gained significant attention as an organic and eco-friendly option for enhancing soil properties and improving crop yields (Zaccardelli, M. et al., 2021). Compost application can improve soil structure, increase water retention, enhance microbial activity, and provide essential nutrients for plant growth.

According to Manohara, B. et al. (2014) solid waste management is a major challenge in urban areas, especially with rapid population growth due to rural-to-urban migration. Increased consumption and industrial activities have led to higher waste generation, and improper disposal contributes to air and water pollution, posing environmental and health risks. The primary goal of urban waste management is to collect, treat, and dispose of waste efficiently and sustainably.

Municipal solid waste (MSW), commonly known as trash or garbage, includes domestic, commercial, institutional, and municipal waste. Proper treatment is crucial, with various traditional and modern methods used depending on waste type. Composting remains a widely practiced method, converting organic waste into a nutrient-rich soil conditioner. However, compost stability is essential, as chemically unstable compost can be harmful to plants due to the release of toxic compounds like ammonia and organic acids.

Many other authors are conducted experiment using municipality compost made from solid waste for growing vegetables. Their findings suggest that, Municipal solid waste compost (MSWC) enhances soil quality by increasing organic matter, which provides several benefits, and boosting total nitrogen, though to a lesser extent. Additionally, MSWC poses a lower risk of nitrate leaching compared to synthetic fertilizers, particularly during the winter season (Cao, X. et al., 2023; Rahman, M. M. et al., 2020; Small G. E. et al., 2024)

Zucchini (*Cucurbita pepo* var. *giromontia*), a widely cultivated vegetable, requires well-balanced soil nutrients to achieve optimal yield and quality. However, conventional farming practices often rely on synthetic fertilizers, which can lead to soil degradation and environmental pollution over time. The use of compost as an alternative or supplement to chemical fertilizers offers a sustainable

approach to improving soil health while maintaining high crop productivity.

This study aims to evaluate the impact of MSWC application on soil properties and zucchini yield. Specifically, it investigates how compost influences soil nutrient content, moisture retention, and biological activity, as well as its effects on plant growth, fruit yield, and quality. By assessing these factors, this research provides insights into the potential benefits of compost in sustainable zucchini production.

MATERIALS AND METHODS

The study took place in 2024 in a suburban region of Sofia city, Bulgaria on the Vrajdebna experimental field hostile to the University of Forestry. The soil type use for the experiment is alluvial-meadow soil.

The compost used as a soil amendment was provided for the experiment by the Sofia Municipality.

Four variants of MSWC treatment were developed, with a tested compost application rate of 5 t/da. The treatments included:

Variant 1 (control) - untreated soil;

Variant 2 - 20% of the optimal compost rate;

Variant 3 - 50% of the optimal rate;

Variant 4 - 100% of the optimal rate.

The optimal rate of compost application for vegetable crops is 5 t/da and was selected based on the literature review (Gravuer, K., & Gunasekara, A., 2016). Each variant was tested in three replicates

Tested crop was *Cucurbita pepo var. giromontia* (zucchini). The preparation of the experimental field and incorporation of soil amendments were conducted during the third decade of March. The amendments were evenly distributed across the experimental plots according to the calculated application rates, followed by plowing to a depth of 15 cm.

Three planting beds were established, where zucchini was sown following the standard double-row belt cultivation system. The sowing took place on May 10, 2024, using a hill-planting method, with a row spacing of 60 cm and 50 cm between plants within the row. The planting arrangement followed a double-row belt system with a spacing scheme of 100+60/50 cm.

The experimental plots were irrigated using a drip irrigation system, with water supply regulated based on the hourly flow rate. The water source for the study was a borehole. No fertilizers were applied during the crop's vegetation period to assess the effects of compost application on plant growth without additional nutrient inputs.

Meteorological data were systematically recorded throughout the experiment. Precipitation levels were measured daily using a standard rain gauge, while average daily temperatures during the crop's vegetation period were documented.

Soil and plant tissue analyses were performed in a certified laboratory following standardized and approved methodologies. Yield assessment was conducted by evaluating the quantity and quality of harvested fruits from ten randomly selected plants per replication.

Before the field experiment was established, samples of the used ameliorant were taken. The compost was obtained from composting urban plant residues and was provided for the purposes of the study by the Sofia Waste Treatment Plant IBT Hhan Bogrov.

Laboratory analyses were performed for the content of essential nutrients and heavy metals. The data from the agrochemical analysis are shown in Table 1.

Table 1. Agrochemical analysis of the imported compost

Total N, %	Total P, g/kg	Fe, g/kg	Zn, g/kg	Mg, g/kg	Ca, g/kg	K, g/kg
1.62	2.183	5.499	0.075	4.309	29.043	7.526
Pb, g/kg	Cu, g/kg	Mn, g/kg	Cd, g/kg	Cr, g/kg	Ni, g/kg	
0.0128	0.0242	0.285	0.0012	0.018	0.007	

The total N content of 1.62% is considered moderate too good for compost, making it a suitable source for improving soil nitrogen content. The phosphorus and potassium content are low compared to plant requirements and is below the recommended levels for compost used as a primary source of phosphorus. Insufficient potassium can limit the effect of potassium application, especially in potassium-loving crops. The compost has a high calcium content of 29.043 mg/kg, indicating a good supply of this element, making the compost suitable for

improving soil structure and balancing pH. The high Ca content is probably due to the use of pH-regulating improvers in the composting process. The compost has a good supply of Mg, but low in P and K, demonstrating its potential as an organic fertilizer. The reported values of iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) are within the limits of the norms for maintaining plant growth.

The content of heavy metals such as lead (Pb – 0.0128 mg/kg), cadmium (Cd – 0.00125 mg/kg), chromium (Cr – 0.018 mg/kg) and nickel (Ni – 0.007 mg/kg) is minimal, confirming the safety of the compost for agricultural use. The analysis shows that the compost is suitable for improving soil fertility, although the relatively low levels of phosphorus, potassium and zinc may require additional fertilization for optimal results.

RESULTS AND DISCUSSIONS

The average monthly air temperature for the growing season in 2024 for the Vrajdebna region is above the average climate norm for the last 30 years.

It has been established that for the months of April and from June to August 2024, the average temperatures exceed the maximum average monthly value for the last 30 years (Figure 1). These higher temperatures in combination with the lower amounts of precipitation outline more extreme conditions for agricultural crops grown without irrigation measures

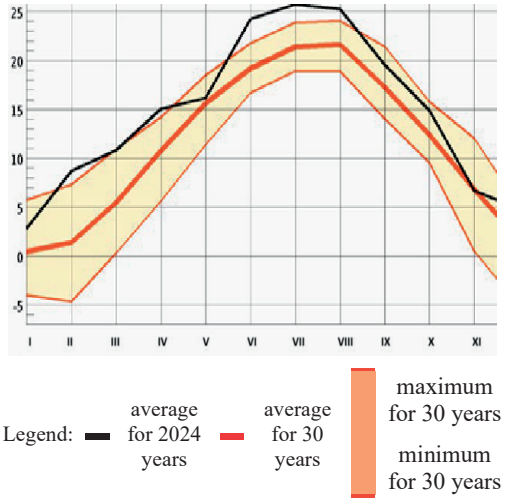


Figure. 1 Average monthly air temperature (^{O}C)

The dynamics of monthly precipitation shows lower amounts of precipitation for the months of the growing season compared to the precipitation for the last 30 years (Figure 2). Only in the months of May and November 2024 did the amounts of precipitation exceed the calculated average climatic norm for 30 years. The longer the blue stripes, the greater the fluctuations in monthly precipitation over the last 30 years, this was found most clearly for the month of May. During the remaining growing months, the amount of precipitation is more constant, with fluctuations over the last 30 years in the range of 50-100 mm. The indicated conditions resuppose the cultivation of more drought-resistant agricultural crops.

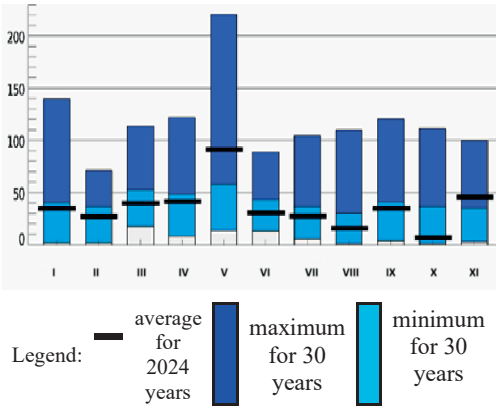


Figure 2. Monthly precipitation (mm)

The average annual precipitation in the city and its surrounding areas is approximately 550-600 mm. A precipitation availability curve was constructed based on a 38-year dataset (Figure 3). Analysis of the obtained data indicates that the year 2024 is characterized by a precipitation availability of 81.51%, classifying it as an average to dry year. In terms of precipitation patterns, 2024 closely resembles the years 2012 and 1998. The total recorded annual precipitation for Sofia in 2024 was 530.9 mm.

Soil nutrient availability plays a crucial role in the growth, development, and yield of zucchini (*Cucurbita pepo* var. *giromontia*). Essential macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), along with secondary and micronutrients, directly influence plant health, fruit quality, and productivity (Rekaby, S. A. et al., 2023).

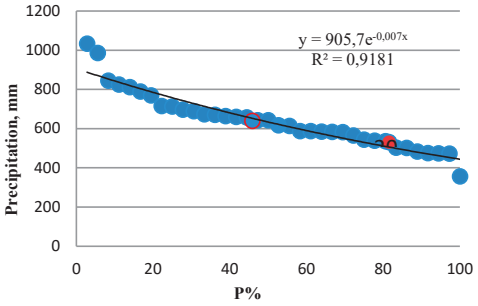


Figure. 3. Curve of precipitation availability for the period 1987-2024

The Table 2 presents data on key soil parameters at a depth of 0-40 cm. The humus content is relatively low, falling below the optimal range of 2-3% typically recommended for vegetable crops. This low humus level may restrict the soil's capacity to retain water and essential nutrients. To enhance soil fertility, the incorporation of organic amendments, such as compost, is advised to increase humus content.

Table 2. Agrochemical characteristics of the soil before planting the zucchini

Depth, cm	Humus, %	pH H_2O	NH_4-N, g/kg	NO3-N, g/kg	P_2O_5, g/100 g	K_2O, g/100 g
0-40	1.73	7.3	0.0413	0.0168	0.046	0.022
Fe, g/kg	**Zn, g/kg**	**Mg, g/kg**	**Ca, g/kg**	**K, g/kg**	**Pb, g/kg**	
21.82	0.048	3.175	4.385	2.115	0.019	
Cu, g/kg	**Mn, g/kg**	**Cd, g/kg**	**Cr, g/kg**	**Ni, g/kg**		
0.032	0.456	0.0015	0.0216	0.0121		

The soil exhibits a neutral pH, which is conducive to zucchini cultivation. A neutral pH optimizes the availability and absorption of both macronutrients and micronutrients, while also fostering beneficial microbiological activity within the soil.

Ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen levels are high, indicating a favorable immediate nitrogen supply for the plants. The presence of both ammonium and nitrate forms suggests a well-balanced nitrogen availability, which is essential for healthy plant growth.

The data on the availability of easily accessible phosphorus indicate a sufficient supply in the soil, supporting the nutrient needs of the crop. Potassium levels in the initial sample are also adequate, ensuring optimal fruit formation for zucchini.

Overall, the soil is in good agrochemical condition for zucchini cultivation. While the low humus content may pose a challenge, the remaining soil indicators - pH, nitrogen, phosphorus, and potassium - fall within the ideal ranges for supporting optimal plant growth.

A higher Fe content is observed, but this is common for soils in urban environments, where it is related to natural origin and the influence of building materials. There are no indications of pollution. The content of heavy metals is very low, with values significantly below the permissible concentrations for urban soils.

The ammonium nitrogen (NH_4-N) content fluctuates across the treatments, with the highest value in Variant 2 (0.0413 g/kg) and the lowest in Variant 3 (0.0147 g/kg) (Table 3). The nitrate nitrogen (NO_3-N) levels remain relatively stable, except for a slight increase in Variant 3. These results suggest that compost application influences nitrogen availability, with excessive nitrogen possibly being subject to leaching or volatilization. Precipitation, combined with maintaining optimal soil moisture, is the likely cause of nitrogen leaching along the profile. Haofeng, Lv. et al. (2021) identified excessive irrigation as the main factor contributing to substantial nitrogen leaching, emphasizing the need for efficient irrigation management strategies.

Available phosphorus content is highest in Variant 4 (0.0545 g/kg), demonstrating the beneficial effect of MSWC application in increasing phosphorus availability. Potassium levels also increase with higher compost rates, reaching a maximum in Variant 4 (0.0268 g/kg) (Table 3).

Iron concentrations remain relatively stable across treatments, with minor fluctuations.

Magnesium levels show minor variations, while calcium content increases with compost application, peaking in Variant 4 (4.421 g/kg). Calcium is vital for cell wall integrity and fruit quality.

The highest calcium content, introduced into the soil through amendments, strongly influences zucchini yield and overall plant development.

Table 3. Agrochemical characteristics of the soil after zucchini harvesting

Variants	NH4-N g/kg	NO3-N g/kg	P2O5	K2O	Fe g/kg
Var.1	0.027	0.0112	0.05	0.0145	21.777
Var.2	0.0413	0.01	0.0464	0.0148	20.857
Var.3	0.0147	0.015	0.0436	0.0212	21.147
Var.4	0.0308	0.01	0.0545	0.0268	21.399
Variants	**Zn g/kg**	**Mg g/kg**	**Ca g/kg**	**K g/kg**	**Pb g/kg**
Var.1	0.049	3.172	4.214	1.964	0.0193
Var.2	0.046	2.946	4.093	1.834	0.0189
Var.3	0.045	2.923	4.005	1.825	0.0146
Var.4	0.048	3.053	4.421	2.002	0.0178
Variants	**Cu g/kg**	**Mn g/kg**	**Cd g/kg**	**Cr g/kg**	**Ni g/kg**
Var.1	0.031	0.457	0.0043	0.022	0.012
Var.2	0.028	0.444	0.0043	0.0205	0.011
Var.3	0.029	0.443	0.0014	0.0199	0.0112
Var.4	0.03	0.447	0.004	0.0207	0.0114

Heavy metal concentrations remain within safe limits, confirming that MSWC application does not pose significant contamination risks.

The obtained data provide insights into the nutrient composition of zucchini fruits across different compost application treatments.

The nitrogen content varies between 2.45% (Var. 3) and 2.91% (Var. 1), indicating that excessive compost application (Var. 3) may have led to a dilution effect, resulting in lower nitrogen accumulation in the fruit. Variant 4, with the highest compost application, shows a relatively high N content (2.89%) (Table 4).

Table 4. Agrochemical characteristics of the zucchini fruits

Variants	Total N%	Total P g/kg	Fe g/kg	Zn g/kg	Mg g/kg	Ca g/kg	K g/kg
Var.1	2.91	7.843	0.119	0.042	1.114	1.071	34.542
Var.2	2.69	6.699	0.107	0.06	1.105	1.169	33.128
Var.3	2.45	7.908	0.083	0.028	1.105	1.352	46.056
Var.4	2.89	7.598	0.129	0.038	1.216	1.42	46.359

The phosphorus content is highest in Var. 1 (7.843 g/kg) and lowest in Var. 2 (6.699 g/kg). Phosphorus is essential for energy transfer and root development, and its reduction in Var. 2 might have impacted plant vigor. The highest compost rate (Var. 3) did not show a significant increase in phosphorus content, which may suggest limited phosphorus availability due to potential nutrient imbalances. The high phosphorus content in the soil is also observed in the fruits.

Calcium levels increase with higher compost application, reaching 1.42 g/kg in Var. 4. This is consistent with findings that compost amendments can enhance calcium availability. However, excessive Ca can interfere with K uptake, which might influence fruit quality. This data for Ca content corresponding to those obtained from soil analyses Table 4.

These results indicate a nutrient imbalance, which directly affects yield.

The yield of zucchini varies greatly. V1 has the highest yield of 6700.94 kg/da, which suggests that the conditions or technology used in this variant are the most effective. In the variant with 20% compost rate, V2 shows 25.4% lower yield than V1, V3 has an even lower yield, 42.1% less than V1. This may indicate increasing limitations in the conditions or factors limiting productivity. V4 has the lowest yield (2807.92 kg/da), which is 58.1% lower than the best variant (V1) (Figure 4).

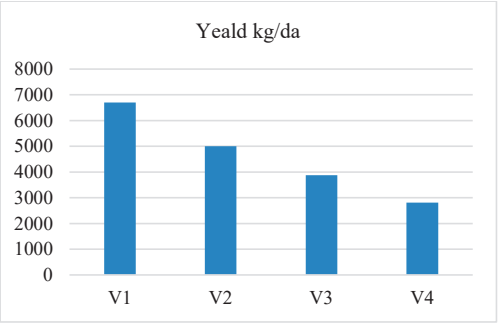


Figure. 4. Yield from field trial with zucchini 2024

The difference was statistically significant (F 3.32=2.94, P<.05).

The same trend is observed when counting the number of fruits harvested, the difference was statistically significant (F 3.36=4.37, P< 0.0099).

However, an excessive calcium supply can interfere with potassium uptake, as previously observed by Bradley and Hosier (1999). Potassium deficiency symptoms, including dull green leaves, chlorotic interveinal spots, and eventual leaf browning and shedding, were evident in Variant 4. This treatment exhibited the most stunted growth, characterized by small, underdeveloped pale green leaves and the lowest recorded yield. These findings highlight the need for a balanced nutrient supply, as excessive calcium application can have adverse effects on plant health and productivity. On the other hand,

when calcium-containing ameliorants are overdosed, the excess affects the absorption of K and Zn. In case of zinc deficiency, spots are observed on the upper leaves, chlorosis and reduced yields, with the yield decreasing by 25%. Similar results have been described by Ma'ruf, A., & Syahminar, S. (2024).

In a greenhouse study on zucchini, M. Tolba et al. (2021) investigated the effects of five different compost application levels. The findings demonstrated a decline in yield with increasing compost rates and revealed a statistically significant linear correlation between total potassium uptake by plants and the dry weight of the fruits.

Monitoring the quality of zucchini, particularly in terms of nitrate accumulation, is essential for consumer safety and public health. Zucchini, like other leafy and fruiting vegetables, can accumulate nitrates under certain growing conditions, posing potential health risks if consumed in excess. According to research by Putnik-Delić, M. et al. (2023), nitrate accumulation in vegetables can result from multiple factors, including the application of nitrogen-based fertilizers, organic matter decomposition, and environmental conditions such as temperature and soil moisture. Proper nutrient management and optimized agricultural practices are crucial for minimizing nitrate buildup, ensuring the production of high-quality and safe zucchini for consumption.

Nitrate levels in all variants were below the permissible values of 200-400 mg/kg, making them safe for consumption. Compost may have contributed to the accumulation of nitrates to varying degrees in the different variants, depending on the application rate and the degree of decomposition of the organic material (Figure 5).

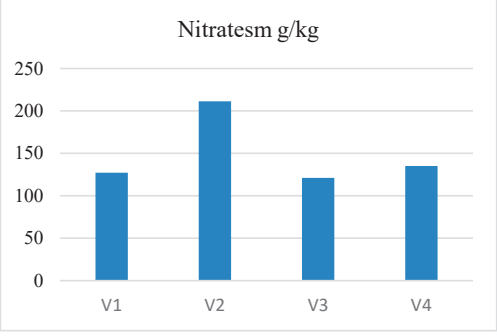


Figure 5. Nitrate content in zucchini fruits

The sugar content in zucchini (*Cucurbita pepo var. giromontia*) fruits is a key indicator of fruit quality, influencing taste, market value, and consumer preference.

Variant 1 has the lowest total sugar content (2.16%), which may be due to more intensive growth and higher yield, which dilute the available sugar reserves in the fruit. V4 shows the highest value (2.83%), which suggests weaker vegetative development or accumulation of more sugars due to stress. In V4, where the yield is lowest, the sugar content is highest (Figure 6).

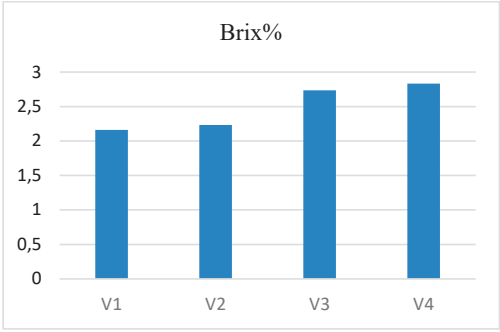


Figure 6. Sugar content in zucchini fruits

The high K content in combination with P is the main reason for the accumulation of more sugars in V4. K is involved in activating enzymes responsible for photosynthesis and starch synthesis, such as sucrose synthase and starch synthase. Higher K availability in V3 and V4 is associated with increased soluble sugars, which enhances fruit taste, sweetness, and consumer preference.

The interaction between potassium and phosphorus plays a crucial role in determining the sugar content and starch accumulation in zucchini fruits.

CONCLUSIONS

The findings of this study demonstrate that the climatic conditions in the studied region are favourable for zucchini cultivation. The urban environment does not contribute to heavy metal contamination of the soil or zucchini fruits, ensuring their safety for consumption.

A thorough understanding of the agrochemical composition of compost prior to its incorporation into the soil is essential for

achieving balanced fertilization and optimizing nutrient availability for vegetable production. The study confirms that increasing compost application leads to higher calcium levels, with a peak of 1.42 g/kg in Variant 4. This aligns with existing research indicating that compost amendments improve calcium availability. However, excessive calcium can negatively affect potassium uptake, which may have implications for fruit development and overall crop quality.

Furthermore, the interaction between potassium and phosphorus is a key factor influencing sugar content and starch accumulation in zucchini fruits. Proper nutrient management strategies are crucial to maintaining an optimal balance between these elements, ensuring high-quality fruit production with desirable taste and texture.

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EFFECT OF INTERCROPPING ON KALE GROWN IN A HIGH TUNNEL FOR WINTER-SPRING PRODUCTION

Gergana MLADENOVA, Milena YORDANOVA

University of Forestry, Faculty of Agronomy, 10 KlimentOhridski Blvd, 1797, Sofia, Bulgaria

Corresponding author email: g.mladenova@ltu.bg

Abstract

The main goal of the experiment was to test the effect of intercropping on kale with other cold-resistant vegetable crops for winter-spring cultivation in a high tunnel. The experiment was conducted on the territory of the experimental field of the University of Forestry - Sofia (42° 7′ N, 23° 43′ E), during the period 2022-2023. Kale, variety "Common Curly", was selected as the main crop, the seedlings of which were planted at a distance of 30x30 cm, during the first ten days of December. As a companion plants were selected: onion, variety "Stuttgarten" (with sets) and lettuce (with seedlings), variety "Lolo rosa". Three variants have been developed: 1) kale - control (KC), 2) kale+onion (K+O), 3) kale+lettuce. (K+L) Biometric measurements were made during the harvest of the produce (April). It was found that in kale, intercropping affects the leaf rosette, leaf size and dry matter content. The plants have a more compact leaf rosette, the leaves are shorter but wider and accumulate a higher percentage (9.5-13.5%) of dry matter compared to the control (8.5%).

Key words*: intercropping, kale, onion, lettice, biometry.*

INTRODUCTION

Kale is considered a superfood with many beneficial properties for human and animal health. It is a rich source of fibers and minerals, prebiotics, unsaturated fatty acids and vitamins, and other biologically active substances in high concentrations (Satheesh and Workneh Fanta, 2020). The plant is tolerant of drought and high temperatures, but it develops best in moderate and low temperatures and regular watering (Cutler, (Ed.). 1995; Naik and Gupta, 2010; Reda et. al., 2021).

The popularity of kale in Bulgaria is growing. It finds a place mainly in private gardens, in urban gardening, as well as in the production range of small family farms, mostly organic, where it occupies a relatively small share of the total production (Silva et al., 2022). In such gardens, it is extremely important to ensure a rich biodiversity for security, sustainability, as a measure against crop failure, plant protection and prevention, and also from an ecological point of view (Marković, 2013).

As a typical representative of the *Brassica* family, kale has limited opportunities to occupy part of the crop rotation for a number of reasons related to the phytosanitary background, soil fertility and economic efficiency (Rana & Mamatha, 2017; Guerena, 2006). Therefore, producers must search for alternative solutions for compaction of areas and crop rotation in time and place.

According to De Pailhe (2014), kale is suitable for inclusion in an intercropping system, especially when its composition is based on the above ground and underground architecture of the plants. In the 2000-2001 project "Companion cropping for organic field vegetables" by Wolfe and Cormack (2002), it was found that kale gave higher yields when grown in mixed crops, along with beets, spinach, and Swiss chard. The team identified these crops as suitable combinations for intercropping, while *Alliacea* crops (onions and leeks) were unsuitable due to the lower yields reported, although their phytoncidal and repellent properties were taken into account.

Intercropping is not considered a necessity, but farms that practice it reap several benefits, at least in terms of plant protection and economic efficiency, as long as it is implemented thoughtfully. It has a strong influence on crop rotation planning (Baldwin, 2006).

When analyzing photosynthesis and carbonization efficiency de Araujo Hendges et

al. (2017), found that kale did not stand out as an aggressive participant in an intercropping system with coriander, Welsh onion, basil, parsley. This experiment is further evidence that the leafy vegetable is suitable for mixed cultivation. Polyethylene greenhouses are used by producers to maximize the time for plants to grow and obtain a harvest (Bruce et al., 2018). Crops that fill the winter months in Bulgaria are usually lettuce, onions, and garlic for greens, radishes, less often arugula, and other leafy greens.

The present study examined how kale behaved when grown alone and in a mixed system with green onions and lettuce, for which no data were found in the scientific literature.

MATERIALS AND METHODS

The experiment was carried out in 2022-2023 in an unheated high tunnel with dimensions of 18.40 by 4.20 m at the Educational-Experimental Field of the University of Forestry in the Vrajdebna district, Sofia, Bulgaria (42° 7′ N, 23° 43′ E).

Kale, variety "Common Curly", was selected as the main crop, the seedlings of which were planted at a distance of 30 x 30 cm in plots 1,50 x 1,50 m in size, during the first ten days of December.

As a companion plants were selected:

1. onion, variety "Stuttgarten" (with sets) planted in the interrows of the kale with a distance of 5 cm between the bulbs.
2. lettuce (with seedlings), variety "Lolo rosso" planted in the interrows, chess-like of the main crop.

Three variants have been developed:

1. kale – control (KC);
2. kale + onion (K+O);
3. kale + lettuce. (K+L);

During the growing season, agrotechnical measures are the same for all variants. They are mainly expressed in watering and weeding. At the beginning, after transplanting the plants until they take root, regular watering is carried out. With a permanent decrease in temperatures in the high tunnel (around 0°C), watering is stopped, which coincides with the development phase of the kale, a rosette with 5-6 leaves.

In the spring, with warming weather, watering is renewed. One weeding is carried out. Fertilization is not carried out. By mid-March, they form an average of 20-25 leaves with excellent taste qualities.

During the last ten days of April, biometric data were taken for all variants - plant height and rosette diameter (10 plants per variant and repetition), leaf length and width (on average 10 leaves per variant and repetition), fresh weight of 10 leaves per variant.

As quality indicators, fresh and absolute dry weight, sugar, and nitrates content were examined. The determination of absolutely dry matter was carried out by weight method in a laboratory dryer at a constant temperature of 60°C until a constant value of the sample was reached. Sugar content (Brix, %) was measured by refractometer, model - Digital refractometer 32145, manufactured by B & C Germany;

Nitrates in leaves were measured by nitrochek meter Boeco.

The collected data were analyzed by ANOVA and were expressed as mean ± standard deviations.

RESULTS AND DISCUSSIONS

Intercropping affects the growth of plants from the main crop - kale, with more pronounced differences in diameter than in plant height. In both variants with intercropping - the combination kale + onion (K + O) and kale + lettuce (K + L) the plants are taller compared to the control (KC), but the differences are not large - on average about 6 cm (Figure 1).

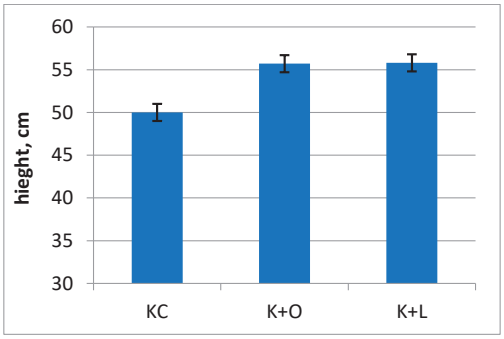


Figure 1. Plant height by variants, cm

The differences in plant diameter are larger - in the variants with intercropping (K + O and K + L) the rosette diameter is smaller compared to the diameter of plants from the control variant

with independent cultivation (Ck), on average about 10 cm (Figure 2). For this indicator, the differences are statistically proven F (2, 36) = 10.15; p <.05.

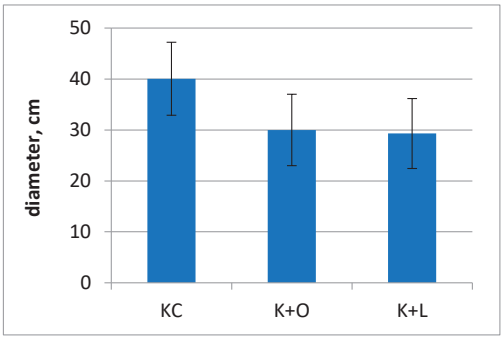


Figure 2. Plant diameter by variants, cm

Those two parameters show that the competetion between the different crops is working and is affecting the plants' architecture above ground.

Also, the spacing between the crops in mono and intercropping systems impacts kale. When kale is grown alone, its average height is low, and its average width is greater. When kale is in an intercropping system, the height is greater, and the width is less.

The influence of intercrops has been studied not only on the whole plant, but also on the leaves, and it is expressed differently in the size of the leaf petioles and the leaf blades.

In the three variants, the length of the leaf petioles is almost the same, with a small difference between those in the intercropping of kale with salads (9.7 cm), in which the leaf petiole is about 2 cm higher (Figure 3).

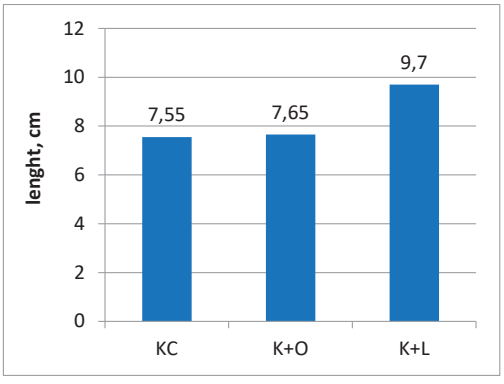


Figure 3. Leaf petioles length by variants, cm

The of the leaf petioles (1 cm) is greater, in the control variant with monocropping compared to those in the variants with intercropping (Figure 4).

Since the differences between the individual variants are relatively small, they are not statistically proven.

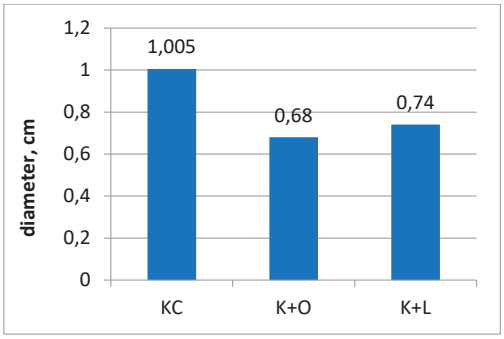


Figure 4. Leaf petioles diameter by variants, cm

In the average results of measuring the width and length of the leaf petioles, intercropping shows a different trend. In terms of leaf petiole length, intercropping with lettuce (K+L) gives better results (17.4 cm) than the other two options, while in intercropping with onion (K+O) the leaf petioles are shorter (16.45 cm), (Figure 5).

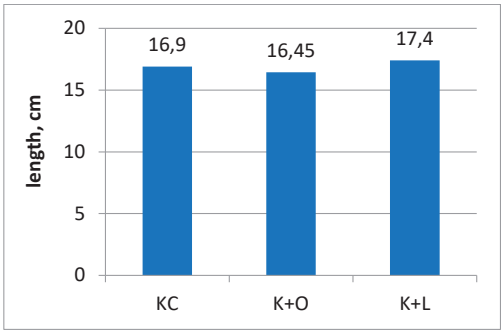


Figure 5. Leaf blade length by variants, cm

The results are similar for leaf blade width. Wider leaf blades (11 cm) for kale when grown in intercropping with lettuce (K+L) and narrower (9.45 cm) when grown in intercropping with onion (K+O). The sizes of the leaf blades in the control variant are close to those of the leaf blades in the variant with intercropping lettuce (Figure 6).

Although the leaf blade sizes of the control variant (KC) are close to those of the kale intercropping with lettuce (K+L), they are inferior in size. The largest are those in the lettuce intercropping, while the leaves in the onion intercropping (K+O) are inferior in size.

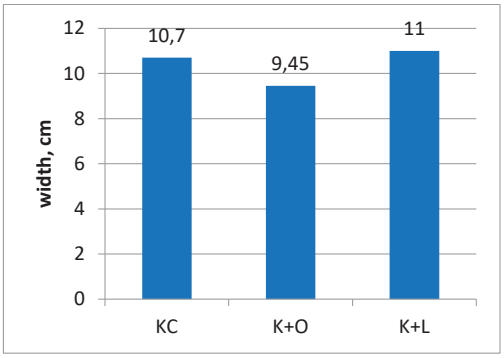


Figure 6. Leaf blades width by variants, cm

During the growing season, the fresh weight of 10 leaves was monitored for the individual variants, as kale is marketed in bundles of 10 leaves or per kilogram.

The data shows that there is very little difference in the weight of the final product when kale is grown alone (KC) compared to when it is grown in intercropping with onions or lettuces (K+O and K+L). The control (KC) and the lettuce variant (K+L) are very close in weight - 62.73 g to 61.78 g. Only the kale + onion variant (K+O) is lighter by 12.59 g than the control (Figure 7).

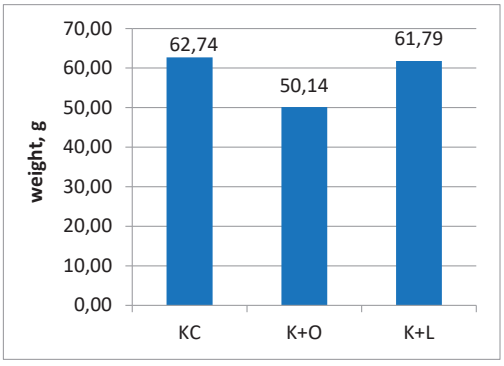


Figure 7. Weight of 10 leaves, g

This indicator shows that growing kale with lettuces does not significantly affect the fresh weight of the leaves, while when growing it with onions, the leaves are slightly smaller and, accordingly, this also affects the average weight of fresh leaves.

Dry matter content is a quality indicator that shows the nutritional value of the plant product, but also how plants use water. In this experiment, the highest dry matter content was observed in kale leaves grown in intercropping with lettuce (K+L) – 13.5%, followed by kale and onion intercropping (K+O) and lastly, the control (KC) (Figure 8).

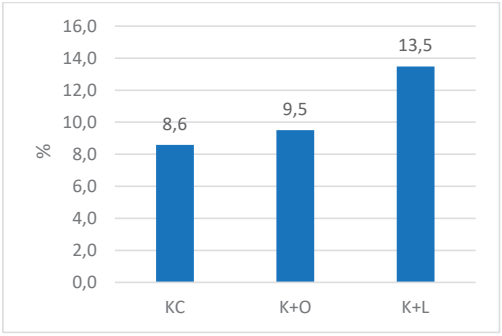


Figure 8. Absolute dry matter, %

There may be several reasons for this:

1. Both kale and lettuce are large consumers of water. The higher dry matter content in kale in this variant may be due to competition between it and lettuce.
2. In the intercropping of kale + onion (K+O), the dry matter in kale is less – 9.5%. The reason for this is most likely due to the biological characteristics of onions to use water rationally, as well as the waxy coating that prevents transpiration losses.

Statistical processing of the data shows that there are proven differences $F(2, 9) = 19.18$; $p < .05$.

Two other quality indicators that were monitored were the content of sugars and nitrates.

As shown in Figure 9, in kale grown in monoculture (KC), the sugar content in the leaves is on average 6.9 brix %.

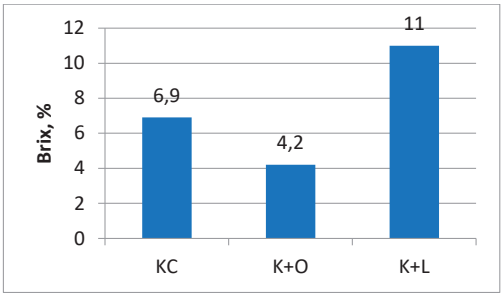


Figure 9. Sugar contend in leaves, brix %

The highest content in this indicator is in the variant kale + lettuce (K+L) – 11 brix %. It is lowest in kale + onion (K+O) – 4.2%.

The explanation of these results is that the higher dry matter content can also lead to a higher concentration of sugars.

Kale is a leafy crop and if grown at low temperatures, it may have a tendency to accumulate nitrates if the nitrogen in the soil is high. According to this indicator, the leaves of all three variants have a low nitrate content, with the results being the same for kale + onion (K+O) – 106 mg/kg and kale control (KC) – 107 mg/kg, and higher data are available for kale + lettuce (K+L) – 186 mg/kg (Figure 10).

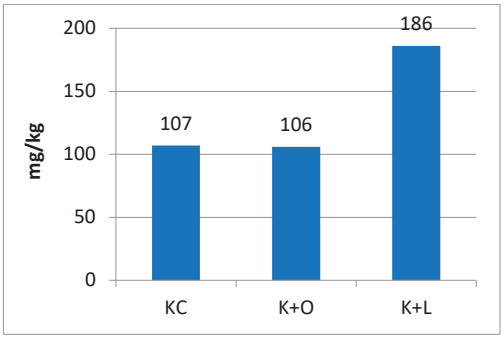


Figure 10. Nitrates contend in leaves, mg/kg

The lower soil temperature, as a result of its denser biomass cover (cabbage and lettuce leaves) and the weak warming in the early spring period, can lead to more difficult absorption of nutrients from the soil, as well as more difficult assimilation of nutrients. This results in a higher nitrate index when growing the kale + lettuce (KL) variant together. However, nitrates are normal.

CONCLUSIONS

Based on the conducted experiment, it can be concluded that the effects of intercropping kale grown in a high tunnel for winter-spring production affected:

1. Rosette height and diameter;
2. Dry matter content;
3. Sugar content;
4. Nitrate content.

It can be confirmed that the competition effect works when kale is grown in intercropping with green onions and lettuce, with a greater effect on qualitative than on quantitative indicators.

Intercropping would have a positive effect on farmers if they grew the three crops together, as in the experiment.

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THE INFLUENCE OF CERTAIN AGROTECHNICAL MEASURES APPLIED IN CARROT TECHNOLOGY UNDER PEDOCLIMATIC CONDITIONS OF VERESTI COMMUNE, SUCEAVA COUNTY

Andreea MORARIU[1], Vasile STOLERU[2], Teodor RUSU[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[2]"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley,
700490, Iaşi, Romania

Corresponding author email: morariu.andreea-sv@ansvsa.ro

Abstract

Carrots are not particularly sensitive regarding cultivation technology but they require increased attention at certain stages. Some of these stages include the preparation of the seed bed, sowing and fertilization. These factors are: the timing and method of seedbed preparation, the sowing scheme, and the method of fertilization. The study was conducted over three years under the pedoclimatic conditions of Veresti Commune, Suceava County. The aim was to develop an innovative technology to increase production. Following the analysis of the data from the three years of study (2016, 2017, 2018) and the comparative analysis of the experimental variants, a significantly positive difference in production was highlighted in the experimental variant where the seedbed was prepared in the fall, the land was shaped, the sowing scheme was 15+15+75+15+15 and fertilization was done with NPK. In this variant, the production increase was 19786 kg/ha, compared to the variant where the seedbed was prepared in the spring, the land was not shaped, the applied sowing scheme was 15+15+75+15+15s, and fertilization was with NPK.

Key words*: fertilizers, seedbeds, quality, production, sowing scheme.*

INTRODUCTION

The focus of Scientific Research has shifted towards development and efficiency to increase productivity. Optimizing agricultural production is a very important and widely discussed aspect, as it is the only sustainable solution to ensure the food supply for the population (Gamez-Vazquez et al., 2022).

Food insecurity has increased in densely populated, urbanized areas with rising incomes. It is expected that these increases in insecurity will be particularly recorded in developing countries with rapid economic growth. All these changes create a favourable environment for the development of farms worldwide, and the food crisis can only be overcome through the involvement of the scientific community.

One of the crops that play an important role nutritionally and has health benefits is the carrot (Stoleru et al., 2016). This plant is among the top ten vegetables cultivated worldwide and represents a significant source of vitamin A (Barbu et al., 2022). After plants in the *Solanaceae* family, such as the potato, carrot roots are the most cultivated for their taste and nutritional properties (Omenn et al., 2005). The carotenoids are must influenced by technological factors as cultivars, fertilizers and irrigation regimes (Stoleru et al., 2020)

Carrots are a plant of significant importance from a dietary, medicinal and industrial perspective. This crop has a high economic yield because it is very productive, and the selling price is attractive for farmers (Mităluț et al., 2021).

The factors that influence production include pedo-climatic conditions, the variety or hybrid, the cultivation technology applied, etc. For example, for producing one ton of vegetable product requird to apply 3 kg N s.a., 1.5 kg P_2O_5 s.a., 5 kg K_2O. The amount of manure recommended for organic fertilization, is 30 t/ha for carrots crop (Dușan & Stan, 2023).

The study aims to observe the influence of innovative elements in carrot cultivation technology with positive effects on production. The experimental factors studied are the type and timing of seedbed preparation, the sowing

scheme, and fertilization (Merlin & Bertrand, 2020).
The purpose of this paper is to study the influence of the period and method of seedbed preparation, the sowing scheme, and fertilization on production.

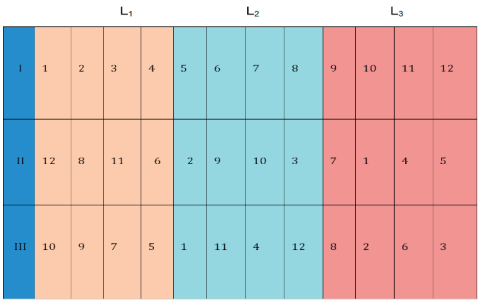
MATERIALS AND METHODS

The study aims to observe the influence of innovative elements in carrot cultivation technology with positive effects on production. Three experimental factors were studied: the first two factors with two levels and the third with three levels, as presented in Table 1.

Table 1. Experimental Factors

Experimental factors	Graduations of the experimental factors
Experimental factor A: Germinal bed preparation	A1 – Conventional seed bed preparation system (the spring, unshaped land)
	A2 – Unconventional seed bed preparation system (the autumn, shaped land)
Experimental factor B: Planting scheme	B1 – Conventional system (planting rows forming strips of 150 cm, distance between rows of 15 cm);
	B2 – Unconventional system (planting, 15 cm+15 cm+75 cm+15 cm +15 cm)
Experimental factor C: Fertilization	C1 – Fertilization with NPK C2 – Fertilization with manure C3 – Fertilization with manure and NPK

The method used to set up the experiment is the Latin rectangle, developed in 1952 by Mudra. This method was employed to study a large number of variants without increasing the number of replications. Mudra divided the columns into sub-columns, which together form a complete column. The number of variants must be divisible by the number of replications. The randomization of variants across columns does not depend on the randomization of variants across blocks; the only condition is that a variant must appear only once per column and also per block. Complete replications allow, within the method, uniform working conditions both in terms of blocks and columns. The large number of variants reduces the value of errors in obtaining results.



Replications: I, II, III; Sub-columns: L1, L2, L3
Experimental Variants: 1, 2, 3, ..., 12

Figure 1. Experimental Plot Layout

The biological material sown over the three years of the experiment is the Laguna F1 hybrid (Figure 2). This is a Nantes-type hybrid with a smooth, somewhat cylindrical root, uniform and very rapid growth. The colour is pleasant, intense orange, and has a commercially appealing appearance.



Figure 2. The hybrid F1 Laguna

In order to determine the physical, chemical, agronomic and morphological properties of the soil, soil samples were collected.
The soil samples were taken along the diagonals of the experimental plot, and laboratory analyses were performed at different depths (0-20 cm, 20-40 cm and 40-60 cm, respectively). Samples were collected on April 6th 2016, April 6th 2017 and April 6th 2018 using a soil auger, from 5 different points along each diagonal of the plot (Rusu, 2005).
The following agrochemical indices were determined: granulometric analysis by the pipette method with result interpretation; pH by the potentiometric method; humus (%) by the Walkley-Black method; total N (%) by the

Kjeldahl method; potassium availability by the flame photometric method; phosphorus availability by the colorimetric method; and base saturation degree (V% = SB / T x 100) (Table 2).

Table 2. Agrochemical indices of the Soil

The bottom (cm)	0-20	20-40	40-60
The coarse sand	0.10	0.09	0.047
The fine sand	46.67	36.03	35.62
The dust I	10.40	18.03	22.79
The dust II	14.92	13.49	8.84
The clay	27.91	32.36	32.70
The interpretation of soil texture	The sandy loam	The loam	The loam
pH	7.68	7.80	7.92
The interpretation	Slightly alkaline	Slightly alkaline	Slightly alkaline
The carbons $CaCO_3$	1.5	1.8	1.80
The interpretation	Slightly carbonated	Slightly carbonated	Slightly carbonated
The humus %	2.15	2.39	1.38
The interpretation	Slightly	Medium	Slightly
Total N %	0.174	0.219	0.187
The interpretation	Moderate	Good	Moderate
The mobile P_2O_5 ppm	40	29	30
The interpretation	Good	Moderate	Moderate
The mobile K_2O ppm	140	116	110
The interpretation	Good	Moderate	Moderate

Carrots are a plant that develops in a temperate climate. Seeds germinate at minimum temperatures of 3-4°C, with a germination period of 20 days at these temperatures. At optimal germination temperatures, between 20-25°C, the germination period shortens to 10 - 12 days (Apahidean and Apahidean, 2000). The optimal temperature for root growth is in the range of 18-22°C. High temperatures favour more vigorous leaf development and result in smaller roots. Root shape is influenced by temperature; lower temperatures and high precipitation favour the formation of sharp, long, and poorly coloured roots. Carrot plants can withstand temperatures as low as -5°C. The vernalisation process occurs at temperatures of 5-10°C over a period of 36-70 days (Indrea et al., 2012).

For normal carrot crop development, soil moisture should be between 65-75% of field capacity during the initial growth period, and then increase to 75-80% during root thickening. The most sensitive periods to moisture are germination, leaf formation, and root thickening (Bender et al., 2009). The climatic parameters in Vereşti commune are similar to those in Suceava city, considering the geographical position relative to the city and the distance of approximately 21 km. Minor differences may be recorded due to the presence of the two rivers, Siret and Suceava.

The year 2016 was climatically erratic, with alternating drought months and months of abundant precipitation. In June and August, reduced precipitation and high temperatures favoured the occurrence of soil drought, while the abundant precipitation in October led to significant losses in agricultural production.

As shown in Figure 3, the maximum temperature from January 1 to December 1, 2016, was recorded at the end of June and exceeded 35°C. The minimum temperature was below -10°C and was recorded in January (Figure 3).

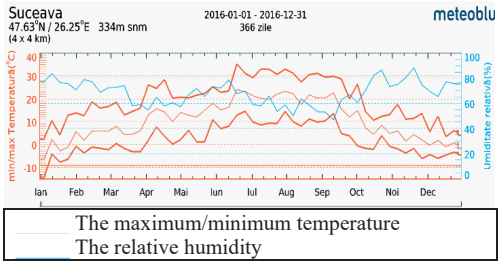


Figure 3. The evolution of air temperature and humidity during January-December 2016
Source: www.meteoblue.com

Precipitation from January 1, 2016, to December 31, 2016, can be observed in Figure 4. In mid-October, the recorded values were over 65 mm, while the smallest amounts of precipitation were recorded in September (20 mm).

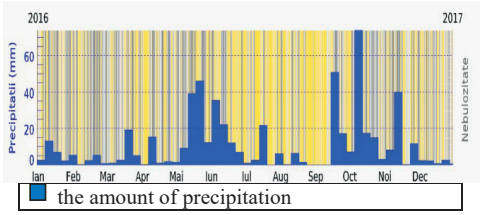


Figure 4. The precipitation evolution during January-December 2016
Source: www.meteoblue.com

In 2017, from the perspective of the most important climatic parameters - temperature and precipitation - the year was relatively erratic. The amount of precipitation was low and uneven. Monthly average temperatures fell within the multiyear averages (8°C), but they varied only in March, July, and August. Large temperature differences between day and night had negative effects on agricultural production.
In the spring, we faced temperature fluctuations and recorded late frosts that affected the carrot crop. During the summer, precipitation was low, and temperatures were high (Figure 5).
The maximum temperature from January 1 to December 1, 2017, was recorded in mid-August and was approximately 40°C. The minimum temperature was recorded in January and was approximately -20°C.

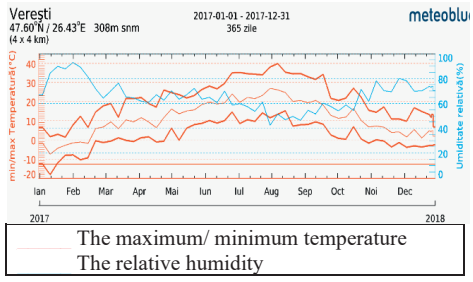


Figure 5. The evolution of air temperature and humidity during January-December 2017
Source: www.meteoblue.com

Precipitation from January 1, 2017, to December 31, 2017, can be observed in Figure 6. The maximum amount of precipitation was recorded in mid-April, exceeding 65 mm, while the minimum amount of precipitation was below 5 mm in mid-October.

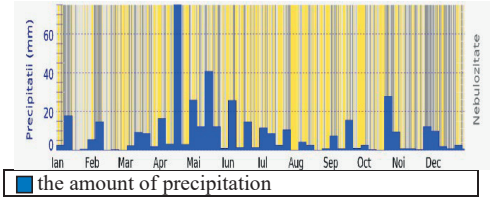


Figure 6. The precipitation evolution during January-December 2017
Source: www.meteoblue.com

In 2018, the year was not as erratic as 2017, and the climatic conditions were favourable for carrot cultivation. Precipitation was adequate and temperatures were moderate. In the spring, we faced reduced precipitation and high temperatures, which was a critical period for soil water availability. There were also temperature fluctuations during this time. In the summer, precipitation was balanced and temperatures were moderate.
The maximum temperature from January 1 to December 1, 2018, was recorded in the latter part of August and was around 35°C. The minimum temperature was recorded at the end of February and was below -15°C (Figure 7).

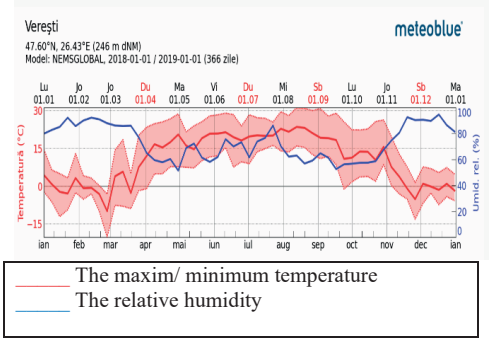


Figure 7. The evolution of air temperature and humidity during January-December 2018
Source: www.meteoblue.com

Precipitation from January 1, 2018, to December 31, 2018, can be observed in figure 8. The maximum amount of precipitation was recorded in mid-June, exceeding 89 mm, while the minimum amount of precipitation was 0 mm from the end of September to the end of October (Figure 8).

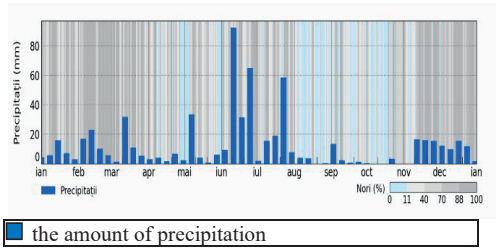


Figure 8. The precipitation evolution during January-December 2018 source: www.meteoblue.com

Observations were made on the development stage of the crop in different phenophases of the carrot plants (Figure 9). The uniformity of the crop and the development of the roots in length and diameter were monitored. The height of the carrot leaves was measured at 10 points of each experimental plot, and samples consisting of 10 carrots were taken to measure the diameter and length of the roots.



Figure 9. The observations in experimental field

The evaluation of agricultural production was determined to establish the quantity. This action was carried out periodically, in phenophases specific to the species, especially after root thickening, 10-20 days before harvest and after harvest.

Production was evaluated through gravimetric measurements, and the obtained results were processed in Microsoft Excel.

The interpretation of the results was carried out using the multi-comparison test method, specifically the Duncan test. This type of test is especially applied in multifactorial experiments. Data processing was conducted using the DSAASTAT.xls database.

RESULTS AND DISCUSSIONS

The results obtained from the experimental field from 2015-2018 are presented in Table 3, where differences in production under the influence of experimental factors can be observed.

Table 3. The productions obtained in 2015-2018

Experimental variants	Repetition I kg/ha	Repetition II kg/ha	Repetition III kg/ha	Arithmetic average kg/ha
a1b1c1	39866	46523	41900	42763
a1b1c2	46400	48113	42166	45560
a1b1c3	34600	35520	36100	35406
a2b1c1	38300	38646	38166	38371
a2b1c2	44916	42450	43066	43477
a2b1c3	37066	37280	46116	40154
a1b2c1	44500	43500	46116	44705
a1b2c2	38836	37906	39233	38658
a1b2c3	63403	44113	43100	50205
a2b2c1	65286	54380	54806	58157
a2b2c2	56833	39336	44723	46964
a2b2c3	46700	43263	42533	44165

As observed in the graphical representation of the yields obtained (Table 3) during the period 2015-2018, the best results were achieved in the experimental variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK. In contrast, the experimental variant V3 yielded the lowest average production per replication, at 35520 kg/ha. In this experimental plot, the seedbed was prepared in the spring, the soil was not ridged, seeds were sown in bands of 150 cm with 15 cm between rows, and fertilization was done with NPK and manure. Good results were also obtained in experimental variants where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK and manure. It can be seen that average yields of over 40000 kg were also obtained in experimental variants where the seedbed was prepared in the spring, seeds were sown in bands of 150 cm width with a distance of 15 cm between rows, and fertilization was done with manure.

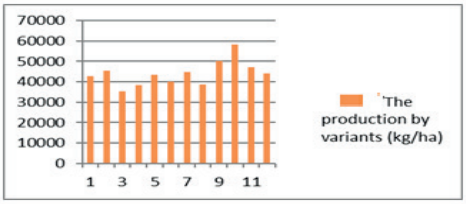


Figure 10. The production by variants, 2015-2018

In Table 4 we can observe the difference of production between variants due to the influence of experimental factors.

Table 4. The comparative interpretation of data - influence of factor A to B and C (The planting seed scheme, the preparation of seeds bed and fertilization)

Variant	Production	%	Difference	Difference of significance
$a_1b_1c_1$	42763	100	0	Mt
$a_2b_1c_1$	44705	104	1942	*
$a_1b_1c_2$	45559	100	0	Mt
$a_2b_1c_2$	38658	84	-6901	0
$a_1b_1c_3$	35406	100	0	Mt
$a_2b_1c_3$	50205	142	14798	***
$a_1b_2c_1$	38370	100	0	Mt
$a_2b_2c_1$	58157	151	19786	***
$a_1b_2c_2$	43477	100	0	Mt
$a_2b_2c_2$	46964	108	3486	-
$a_1b_2c_3$	40154	100	0	Mt
$a_2b_2c_3$	44165	110	4011	-
DL (p 5%) = 435 kg/ha; DL (p 1%) = 898 kg/ha; DL (p 0.1%) = 1082 kg/ha;				
*positive not significant difference 0 - negative not significant difference ***distinctly highly significant positive difference - not significant difference				

As we can observe (Table 4), the best results in terms of production were obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK. Compared with the variant where the seedbed was prepared in the spring, while the sowing scheme and fertilization were carried out in the same manner as in the previously mentioned variant, a production increase of 19786 kg was recorded. Distinctly highly significant positive results were also obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown in bands of 150 cm with a distance of 15 cm between rows, and fertilization was done with NPK during the growing season, with manure applied during seedbed preparation.

In Table 5 we can observe the difference between variants generated by experimental factors.

Table 5. The comparative interpretation of data's - influence of factor B to A and C (The planting seed scheme, the preparation of seeds bed and fertilization)

Variants	Production	%	Difference	Difference of significance
$b_1a_1c_1$	42763	100	0	Mt
$b_2a_1c_1$	38370	89	-4392	00
$b_1a_1c_2$	45559	100	0	Mt
$b_2a_1c_2$	43477	95	-2082	0
$b_1a_1c_3$	35406	100	0	Mt
$b_2a_1c_3$	40154	113	4747	*
$b_1a_2c_1$	44705	100	0	Mt
$b_2a_2c_1$	58157	130	13452	***
$b_1a_2c_2$	38658	100	0	Mt
$b_2a_2c_2$	46964	122	8305	*
$b_1a_2c_3$	50205	100	0	Mt
$b_2a_2c_3$	44165	88	-6040	-
DL (p 5%)= 731 kg/ha; DL (p 1%)= 1061 kg/ha; DL (p 0.1%)= 1620 kg/ha;				
*positive not significant difference ***distinctly highly significant positive difference 0 - negative not significant difference 00 - significantly negative difference - not significant difference				

From the comparative analysis of the experimental factors B, A, and C, we can observe that the highest yield was obtained in the experimental variant where the sowing scheme was 15+15+75+15+15, the seedbed was prepared in the fall, the soil was ridged, and fertilization was done with NPK. In this variant, the production difference is distinctly highly significant positive, with an increase of 30%, compared to the variant where the sowing scheme was in bands of 150 cm with a distance of 15 cm between rows, fertilization was done

with NPK, and the same seedbed preparation method was used. Thus, we can recommend that the carrot crop be sown according to sowing scheme 15+15+75+15+15, the seedbed be prepared in the fall, the soil be ridged, and fertilization be done with NPK.

Table 6. The comparative interpretation of dates - influence of factor C, A to B (The fertilization method, preparation of seeds bed and planting scheme)

Variant	Production	%	Difference	Difference of significance
$c_1a_1b_1$	42763	100	0	Mt
$c_2a_1b_1$	45559	107	2796	*
$c_3a_1b_1$	35406	83	-7356	00
$c_1a_1b_2$	38370	100	0	Mt
$c_2a_1b_2$	43477	114	5106	**
$c_3a_1b_2$	40155	115	1783	*
$c_1a_2b_1$	44705	100	0	Mt
$c_2a_2b_1$	38658	87	-6047	0
$c_3a_2b_1$	50205	112	5500	**
$c_1a_2b_2$	44165	100	0	Mt
$c_2a_2b_2$	46964	106	2798	*
$c_3a_2b_2$	58157	131,68	13992	***
DL (p 5%)= 1421 ha/kg; DL (p 1%)= 1622 kg/ha; DL (p 0.1%)= 2472 kg/ha;				
*positive not significant difference ***distinctly highly significant positive difference 00 - significantly negative difference - not significant difference				

A comparative analysis of the results obtained in the experimental variants under the influence of the gradations of experimental factor C, in relation to factors A and B, showed that the highest production was achieved in the experimental variant where fertilization was done with NPK and manure, the seedbed was prepared in the fall and ridged, and sowing was done according to scheme 15+15+75+15+15. The production differences were distinctly highly significant positive compared to the variant where fertilization was done with NPK and the seedbed was prepared in the fall, ridged, and the sowing scheme was 15+15+75+15+15. It is recommended to fertilize the carrot crop with manure and NPK, provided that the seedbed is prepared in the fall, the soil is ridged, and sowing is done according to scheme 15+15+75+15+15. Distinctly significant negative differences were recorded in the variant where fertilization was done with NPK and manure, the seedbed was prepared in the spring, the soil was not ridged, and sowing was done in bands of 150 cm with a distance of 15 cm between rows.

Table 7. The synthesis of the comparison through the Duncan test

No.	Variants	Production	Classification
1	$a_1b_1C_3$	35406	A
2	$a_1b_2C_1$	38370	AB
3	$a_2b_1C_2$	38658	ABC
4	$a_1b_2C_3$	40154	ABC
5	$a_1b_1C_1$	42763	ABCD
6	$a_1b_2C_2$	43477	ABCD
7	$a_2b_1C_3$	44165	BCD
8	$a_2b_2C_1$	44705	BCD
9	$a_1b_1C_2$	45559	BCD
10	$a_2b_2C_2$	46964	CD
11	$a_2b_1C_3$	50205	D
12	$a_2b_2C_1$	58157	E

In the interpretation of the data using Duncan's test, it can be observed that variants with similar influences and productions are marked with common symbols in the classification column. Thus, there are no significant differences between the variants where the seedbed was prepared in the spring, sowing was done in bands of 150 cm with a distance between rows of 15 m, and fertilization was done with manure, and the variants where the seedbed was prepared in the spring, the soil was not ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK and manure. Comparing the results from all experimental variants, it can be observed that significant production differences were obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. On the opposite end, with a production of 35406 kg and significant negative results, is the variant where the seedbed was prepared in the spring, the soil was not ridged, sowing was done in bands of 150 cm with a distance of 15 cm between rows, and fertilization was done with NPK and manure. In recent years and especially at the U.S.A.M.V. Different studies were carried out in Cluj-Napoca with the aim of improving

carrot culture technology. Bota, during three years, studied the optimal time for seeding and fertilization specific to root vegetable crops, including carrot crops. Mănuțiu, he studied the behaviour of 2 carrot hybrids (FLAKKER-3, NANTES-5), in the period 2010-2012, under the influence of sowing time and facial chemical fertilization. The best production results were obtained for the Flakker-3 hybrid, in the first sowing season (March) with an increase of 5.08 t. He found that the Fakker-3 hybrid reacted favourably (35.30 t), when sown in the first stage (March) and chemical phase fertilization, while Nantes-5 hybrid reacted favourably to manure fertilization (29.02 t) and sown in the same period (March).

The productions obtained in the other experimental variants are close in values. The linear regression of production, based on the interaction of the experimental factors, shows a uniformly upward trend across the entire graphical representation.

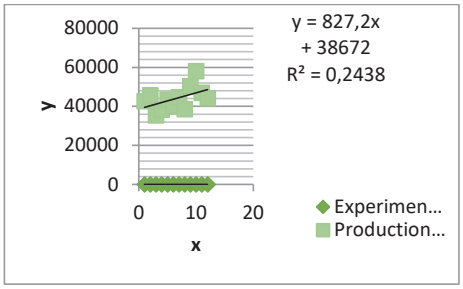


Figure 11. The linear regression of production

From the graphical representation, it can be observed that production can either increase or decrease depending on the method of seedbed preparation. It is noted that if the seedbed is prepared in the fall, the soil is ridged, sowing is done according to scheme 15+15+75+15+15, and fertilization is done with NPK, the production difference is distinctly highly significant positive, compared to the variant where the seedbed is prepared in the spring, the soil is not ridged, sowing is done in bands of 150 cm with a distance between rows of 15 cm, and fertilization is done with NPK.

CONCLUSIONS

Thus, based on the three years of studies and the comparative analysis of the experimental variants under the influence of the three experimental factors, a distinctly highly significant positive production difference is highlighted in the experimental variant where the seedbed was prepared in the fall, the soil was ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. In this variant, the production increase is 19786,66 kg/ha, compared to the variant where the seedbed was prepared in the spring, the soil was not ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. Therefore, it is recommended to prepare the seedbed in the fall, ridge the soil, sow according to scheme 15+15+75+15+15, and fertilize with NPK. The other variants yielded significant positive results with similar values.

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BIOLOGICAL POTENTIAL OF A COMMON BEAN COLLECTION IN RELATION WITH THE CLIMATE CHANGE

Valko NESHEV

Agricultural Academy, Institute of Plant Genetic Resources "Konstantin Malkov" – Sadovo, 2 Druzhba Str., 4122, Sadovo, Plovdiv District, Bulgaria

Corresponding author email: vlknshv@yahoo.com

Abstract

Common bean is a traditional grain legume crop in Bulgaria. It is mostly grown and used worldwide for its edible seeds. The genebank at IPGR-Sadovo preserves a large number and diverse collection of Phaseolus vulgaris L. Biotic and abiotic stress factors are causing significant yield and quality losses in common beans. The study aims to investigate accessions by biological, morphological, and agronomic traits and to select genotypes with diseases and drought resistance. For the current study, 26 accessions of local and foreign origin were used. Most of them are traditional varieties. The biometry and phenology analyses follow the Descriptors for Phaseolus vulgaris of IBPGR (1982). Observations on resistance to bacterial disease symptoms, caused by the pathogen, Xanthomonas campestris pv. Phaseoli were made using the CIAT scale (1987). The accessions were grown under nonirrigated conditions and the drought tolerance was recorded. The study gives valuable preliminary information for the common bean collection's biological potential about climate change. The investigation will be continued as a complex evaluation in the next experimental years.

Key words*: genebank, common bean, agro-climatic conditions, biometry, disease resistance.*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) originates from Asia and the tropical regions of America. It is considered one of the oldest cultivated plants. American types of beans are commonly grown in Europe (Agricultural Academy, 1973).

The largest producers of beans in the world are India, Brazil, Mexico, and the European countries – Turkey, Romania, Italy and France (Ivanova et al., 2019). This species is an excellent precursor for almost all field crops because it enriches the soil with nitrogen.

The beans from *Phaseolus vulgaris* L. are mainly used for culinary purposes. Various traditional dishes are prepared from its seeds, such as purees, soups, stuffed peppers, etc. Both dry and green beans are used for canning. It is widely consumed in its green state when the vitamin C content is higher. Bean seeds are high in protein, fats, carbohydrates, minerals, B vitamin, and provitamin A (Ivanova et. al., 2019). Because of this, the quality of the seeds and pods is one of the important traits, which is also related to the resistance of the plants to drought and diseases.

Except for the common bean, there are many other traditional grain legume crops worldwide, and collecting plant germplasm is essential for saving biodiversity and its use in breeding and practice. Plant genetic resources are a national treasure that must be sustainably conserved and used (FAO, 2014).

The main priority of the scientific team of IPGR "K. Malkov" – Sadovo, part of the Agricultural Academy – Bulgaria, is the long-term storage of plant germplasm through seeds under controlled conditions in the National Genebank. IPGR-Sadovo is part of the European Program on Plant Genetic Resources (ECPGR, 2021) and the collection is published with open access in the European catalogue EURISCO (http://eurisco.ecpgr.org).

The yields of major agronomic crops are negatively impacted by climatic changes in several aspects as the intensity of rainfall, increasing the mean temperature, heat waves, changes in weeds, disease-causing microorganisms, and pest attacks during all growing seasons. Higher temperature frequently causes a reduction in crop yield because, they generally happen in combination with drought.

Crop phenology is negatively affected due to the climate change (Fatima et al., 2021).
The aim of the study is to investigate the collection by biological, morphological, and agronomic traits and to select accessions with diseases resistance and drought tolerance.

MATERIALS AND METHODS

The comparative experiment in the field of IPGR-Sadovo, Department of Plant Genetic Resources was conducted.
Twenty-six accessions, including 17 acc. with local Bulgarian origin (12 acc. determinant and 5 acc. indeterminate) and 9 acc. with diverse origin (USA – 1 acc.; Korea – 1 acc.; Germany – 2 acc.; Hungary – 1 acc.; Portugal – 1 acc.; Turkey – 1 acc.; Chilly – 2 acc.), from which 7 determinant and 2 indeterminate accession were planted (Table 1).
Each of the accessions was grown in two replications in two row plots with a length of 2 m.

Table 1. List of the accessions used for the study

N	Cat. N	Taxonomy	Growth habit	Origin
1	A9E1426	*Ph. vulgaris* L.	determinate	Local/BGR
2	A9E0639	*Ph. vulgaris* L.	determinate	Local/BGR
3	A9E0658	*Ph. vulgaris* L.	determinate	Local/BGR
4	A9E1101	*Ph. vulgaris* L.	determinate	Local/BGR
5	A7E0668	*Ph. vulgaris* L.	determinate	Local/BGR
6	C1E0039	*Ph. vulgaris* L.	determinate	Local/BGR
7	C2E0025	*Ph. vulgaris* L.	determinate	Local/BGR
8	C0E0310	*Ph. vulgaris* L.	determinate	Local/BGR
9	C4E0001	*Ph. vulgaris* L.	determinate	Local/BGR
10	C4E0002	*Ph. vulgaris* L.	determinate	Local/BGR
11	C4E0003	*Ph. vulgaris* L.	determinate	Local/BGR
12	C4E0004	*Ph. vulgaris* L.	determinate	Local/BGR
13	A9E0405	*Ph. vulgaris* L.	indeterminate	Local/BGR
14	A9E0608	*Ph. vulgaris* L.	indeterminate	Local/BGR
15	A9E0182	*Ph. vulgaris* L.	indeterminate	Local/BGR
16	A7E0137	*Ph. vulgaris* L.	indeterminate	Local/BGR
17	84201011	*Ph. vulgaris* L.	indeterminate	Local/BGR
18	91201180	*Ph. vulgaris* L.	determinate	USA
19	91201083	*Ph. vulgaris* L.	determinate	KOR
20	69886	*Ph. vulgaris* L.	determinate	DEU
21	91201122	*Ph. vulgaris* L.	determinate	DEU
22	771423	*Ph. vulgaris* L.	determinate	HUN
23	91201025	*Ph. vulgaris* L.	determinate	PRT
24	91201228	*Ph. vulgaris* L.	determinate	TUR
25	771427	*Ph. vulgaris* L.	indeterminate	CHL
26	771458	*Ph. vulgaris* L.	indeterminate	CHL

A conventional cultivation method was used, which included several treatments (manual and machine), as well as plant protection and fertilization of crops. All the accessions were grown under nonirrigated conditions. The agro-climatic conditions are monitored by a meteostation, located in the IPGR-Sadovo.
For the biometry and phenology observations, the Descriptors for *Phaseolus vulgaris* (IBPGR, 1982) was used.
Phenology measurements were carried out during the growing season of the crop, recoding the following phenology periods: days to germination – the days from sowing to the germination of 50% of the plants under the surface; days to flowering – number of days from germination to stage where 50% of plans have set flowers; duration of flowering – number of days from first flowers in 50% of the plants to the stage when 50% of the plants have stopped flowering; days to maturity – number of days from emergence until 90% of the pods are mature.
For the biometric analyses, 20 randomly chosen plants per replication. The biometry traits are: plant height; number of pods per plant; beans per pod; the weight of 1000 seeds; seed (length, width, and thickness); length of the pod; height of the first pod. An average sample of 20 pods and 20 seeds was taken and biometry was measured using Mitutoyo 500-196-30 Digimatic Absolute Caliper 150 mm. The weight of 1000 seeds was measured using a digital scale.
For evaluating the plant's reaction to drought, a scale of 1-3 was used for sensitivity to stress from the Descriptors for Phaseolus vulgaris (IBPGR, 1982), where 1 is slightly sensitive, 2 is medium sensitive, and 3 is highly sensitive for symptoms such as loss of turgor of the leaves, dry plants, etc.
Visual investigation of typical for the bacterial disease “common bacterial blight” symptoms was conducted. The severity of the symptoms was evaluated using a 1-9 scale, where 1 - no visual symptoms, 3 - symptoms are observed on 2% of the leaf blade surface; 5 - symptoms are observed on 5% of the leaf blade, diameter of the spots - 5 mm; 7 - symptoms are observed on 10% of the leaf blade, diameter of the spots - 10 mm; 9 - symptoms are observed on more than 25% of the leaf blade (CIAT, 1987; Genchev and Kiryakov, 2005).

The created characterization and evaluation database from the experiment was statistically processed using IBM SPSS Statistics 26 software and Analysis Tool Pak in MS Excel. The variation is considered as weak if the coefficient of variation (CV %) does not exceed – 0%, medium - the variation between 10-20%, and strong (significant) – when the variation is over 20% (Genchev et al., 1975; Dimova and Marinkov, 1999).

RESULTS AND DISCUSSIONS

The temperatures during the growing season were higher compared to the temperature norms for the region of Sadovo, Central South Bulgaria. In the experimental period, the maximum temperatures during the bean formation ranged from 33.7ºC to 36.4ºC (Figure 1). The minimum temperatures ranged from 8.7ºC in April to 18.4ºC in July.

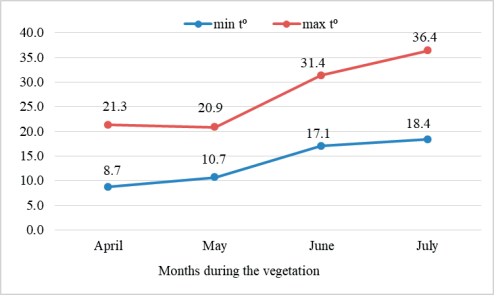


Figure 1. Min and Max temperature from sowing to harvesting of common bean

Precipitation is also one of the most important factors that determines the yield in our country. For the seed germination, it needs to absorb 80-145% water by weight (Ivanova et al., 2019). The sum of the fallen rains during the vegetation of the plants was 123.6 l/m^2. The highest quantity of rain was recorded in May during the plant's development (Figure 2). It has to be noted that most of the precipitation in May fell after the appearance of the first plants. Also, the amount of precipitation in June is scarce.

After the sowing date, the first sprouted plants were noted in May, which is quite a long period. The periods that are compared are presented in Table 2, where: days to germination – the days from sowing to the germination of 50% of the plants under the surface; days to flowering – the number of days from germination to stage where 50% of plans have set flowers; duration of flowering – number of days from first flowers in 50% of the plants to the stage when 50% of the plants have stopped flowering; days to maturity – number of days from germination until 90% of the pods are mature.

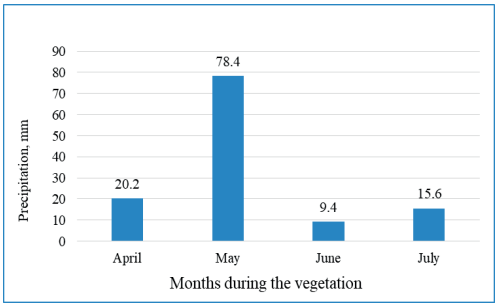


Figure 2. The sum of a precipitation by months from sowing to harvesting of common bean

The days of the germination period are noted for three accessions with longer periods (28 days): 91201122; 771423 and 771427 from the introduction. One day earlier A9E1101; C1E0039; C2E0025; C4E0002; C4E0004; A9E0182; 84201011; 91201180; 91201083; 771458 are germinated. With the shortest duration period of 25 days to germination are the accessions: A9E1426; A9E0639; A9E0658; A7E0668; C0E0310; C4E0001; C4E0003; A9E0405; A9E0608; A7E0137; 69886; 91201025 and 91201228. With the longest period to flowering is A9E0608 from the expedition and the earliest are C4E0004; 91201083; 91201122; and 771423. According to days to the duration of flowering, there are noted A9E0658; C4E0004 with 26 days and with the shortest duration (15 days) are A9E0608 and 771458. About the vegetation cycle could be said that with shortest period are 91201122 and 771427; with the longest period are 91201025 and 91201228. There is no big difference between the vegetation periods of the accessions. The variation is only between 5 days.

If we compare the close results in the duration of vegetation: the short vegetation period, the duration of flowering, and days to germination which is quite a short period too, it could be assumed that the high maximum temperatures and the fewer precipitations in the important stages of growing are negative factor for the

common bean. The lack of rainfall under nonirrigated is considered critical. As a result, in these conditions, the vegetation of all accessions was recorded as delayed and late germination. The rains were scarce and the plants did not germinate uniformly (Photos 1 and 2).

In common beans (*Ph. vulgaris* L.) high temperatures affect negatively flowering, fruit setting, and maturity cycle, such as stress factors leading to the abortion of flowers and pods, and causing premature ripening of plants (Minoz-Perea et al., 2006).



Photos 1 and 2 – Germination of the studied collection

Table 2. Phenological data for the experiment

N	Cat. N	Days to germination	Days to flowering	Duration of flowering	Days to maturity
1	A9E1426	25	32	17	71
2	A9E0639	25	37	22	71
3	A9E0658	25	29	26	73
4	A9E1101	27	30	17	69
5	A7E0668	25	30	25	71
6	C1E0039	27	31	24	69
7	C2E0025	27	30	24	69
8	C0E0310	25	31	25	71
9	C4E0001	25	30	22	71
10	C4E0002	27	30	21	69
11	C4E0003	25	30	18	71
12	C4E0004	27	27	26	69
13	A9E0405	25	32	18	71
14	A9E0608	25	34	15	71
15	A9E0182	27	31	17	69
16	A7E0137	25	30	22	71
17	84201011	27	28	21	69
18	91201180	27	28	25	69
19	91201083	27	27	21	69
20	69886	25	30	22	70
21	91201122	28	27	22	68
22	771423	28	27	22	67
23	91201025	25	32	24	73
24	91201228	25	32	22	73
25	771427	28	31	22	68
26	771458	27	32	15	71

For characterizing the studied accessions biometric measurements were conducted (Table 3). Plant height and the height of the first pod are important traits for grain quality, whereas pods located in the upper part of the plant will avoid contact with the soil surface, which is a source of pathogens, according to Dianatmanesh et al. (2017).

In the studied group the average plant height is 38.45 cm. The accessions with the highest plants are: A9E0182; A9E0405 and A9E0405 but with lowest are 771423; 69886 and 771427. With the highest pod position are the accessions A9E1426 and C4E0002. Accessions with more beans per pod are: 771427; 91201228; 91201122; and C2E0025. The weight of 1000 seeds is an important trait and the mean from Table 3 is 200 g. With a maximum weight of 1000 seeds is distinguishes 91201180 of 255 g and A9E0182; A9E0405; C4E0001 follow him with around 240 g. The trait number of pods per plant is maximum presented in the accession 771458 from Chilly with the maximum number of pods per plant (9.00) and with a minimum is

C4E0004 (local variety pinto bean). The maximum length of the pod is cat. N 91201083. The traits of the seeds – length, width, and thickness are very variable. The mean scales for the seed sizes are: length - 1.12 cm, width - 0.64 cm, and thickness - 0.5 cm.

Table 3. Biometric measurements conducted for characterizing the studied accessions

N	Cat. N	Plant height, cm	Number of pods per plant	Beans per pod	Weight of 1000 seeds	Seed length	Seed width	Seed thickness	Length pod	Height of the 1st pod
1	A9E1426	39.80	3.50	3.00	195	1.18	0.61	0.50	7.50	23.50
2	A9E0639	31.30	2.50	2.60	105	0.82	0.60	0.46	6.00	16.20
3	A9E0658	44.80	3.00	2.60	190	0.87	0.69	0.48	7.20	180
4	A9E1101	30.40	4.70	2.60	225	1.22	0.79	0.58	8.20	16.20
5	A7E0668	31.40	3.60	2.00	165	1.07	0.64	0.47	6.40	16.00
6	C1E0039	34.10	2.00	2.00	205	0.96	0.65	0.62	9.10	14.30
7	C2E0025	36.50	6.00	4.00	195	0.98	0.69	0.50	8.10	20.40
8	C0E0310	39.60	4.00	2.50	155	1.14	0.56	0.52	7.70	17.70
9	C4E0001	34.00	3.80	2.60	240	1.28	0.61	0.48	9.60	20.50
10	C4E0002	42.60	2.30	2.00	220	1.22	0.68	0.49	7.50	23.50
11	C4E0003	36.20	2.60	2.40	195	1.09	0.73	0.46	6.50	20.20
12	C4E0004	34.80	1.50	3.00	205	0.97	0.63	0.45	8.70	19.10
13	A9E0405	57.70	3.80	3.00	245	0.95	0.76	0.49	6.50	15.60
14	A9E0608	47.70	2.00	1.30	155	1.17	0.69	0.53	6.70	21.00
15	A9E0182	60.80	3.00	2.80	245	1.13	0.56	0.54	7.10	22.5.
16	A7E0137	37.60	1.70	2.00	220	1.18	0.64	0.50	5.50	16.30
17	84201011	57.80	6.60	2.00	195	1.15	0.66	0.50	8.00	17.50
18	91201180	31.00	2.20	2.20	255	0.94	0.67	0.57	6.60	16.20
19	91201083	33.00	3.60	1.80	239	1.20	0.58	0.48	12.20	17.00
20	69886	27.00	3.70	3.50	187	1.05	0.60	0.53	9.30	12.50
21	91201122	31.50	3.00	4.20	146	1.04	0.55	0.40	7.50	15.30
22	771423	25.10	4.00	1.50	217	1.17	0.57	0.57	8.80	12.00
23	91201025	41.20	3.20	3.20	181	1.16	0.58	0.39	9.30	20.30
24	91201228	36.20	3.00	4.50	173	1.83	0.59	0.40	11.60	22.70
25	771427	28.70	5.50	4.30	213	1.26	0.62	0.50	7.70	12.00
26	771458	49.00	9.00	2.50	233	0.98	0.70	0.50	9.10	17.60
	Mean	38.45	3.61	2.70	200	1.12	0.64	0.50	8.02	17.85
	Min	25.10	1.50	1.30	105	0.82	0.55	0.39	5.50	12.00
	Max	60.80	9.00	4.50	255	1.83	0.79	0.62	12.2	23.5
	St. error	1.87	0.33	0.17	7.00	0.04	0.01	0.01	0.31	0.66
	St. deviation	9.55	1.67	0.85	35.72	0.19	0.06	0.06	1.59	3.36
	CV %	24.84	46.26	31.48	17.86	16.96	9.38	12.00	19.83	18.82

The coefficient of variation (CV %) is an important statistical feature that makes it possible to establish the relative alignment of the variation among the studied group. It is also important to know how much the studied material is level or heterogeneous and the compared signs are variable. Taking into account the coefficient of variation, the variation is strongest in the following indicators: height of the plant, number of seeds per pod; pods per plant. The variation is medium in the weight of 1000 seeds; seed length; seed thickness; length of pod and height of the first pod. The variation was considered as low only for the trait seed width.

In the agro-climatic conditions the reaction of the plants to drought and diseases was studied. For the disease evaluation visually were monitored the symptoms on leaves typical for "common bacterial blight". Among the accessions with local origin that did not show symptoms of the infection are: C0E0310; C4E0001; C4E0002; C4E0003 and 84201011, and those of foreign origin are: 91201083 (KOR); 69886 (DEU); 91201122 (DEU); 771423 (HUN); 91201025 (PRT); 91201228 (TUR); 771427(CHL) and 771458 (CHL) (Figure 3).

As a result of this preliminary evaluation the local traditional varieties C0E0310, C4E0001,

C4E0002, C4E0003 and the German 69886, showed good resistance to both stress factors – drought and the disease.

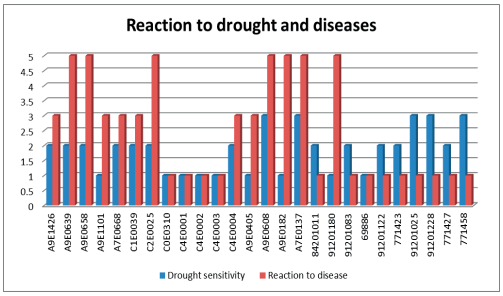


Figure 3. Reaction of the plants to drought and diseases

On the basis of all evaluated traits the accessions were classified by hierarchical cluster analyses using average linkage (Between groups) (Figure 4). According the dendogram the studied groups of accessions could be divided in 3 clusters. In all of them are grouped accessions with different origin. Similar results are presented from Stoilova et al. (2024). In the first cluster are grouped 14 accessions (from 1 to 14). In the second cluster are included 11 accessions (from 10 to 26). These genotypes are characterized with larger seeds compared to the accessions grouped in the cluster 1. As the most different from all accessions is the cat. N A9E0639 (2). This genotype is not grouped with other forms and is characterized with the smallest seeds and high sensitivity to drought and disease.

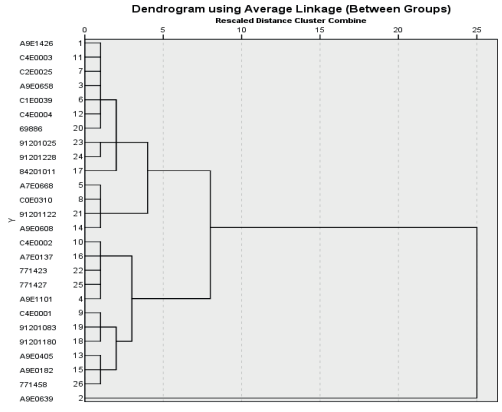


Figure 4. Dendogram of the clusterisation using Avarage Linkage (Between Groups)

CONCLUSIONS

As a result of this preliminary evaluation the local Bulgarian varieties C0E0310, C4E0001, C4E0002, C4E0003 and the German accession 69886, showed good resistance to both stress factors – drought and the disease.

According the analysis the studied collection could be divided in three clusters. In each of them the accessions are with diverse geographical origin.

The most different from all accessions is the local Bulgarian genotype A9E0639. It is characterized with the smallest seeds and high sensitivity to drought and disease and should be excluded from future breeding schemes aiming at resistance and good seed quality.

For further evaluation more precise experiments are needed.

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EVALUATION OF PHYSIOLOGICAL PROCESSES IN SOME POTATO GENOTYPES CULTIVATED ON SANDY SOILS IN SOUTHERN OLTENIA

Alina-Nicoleta PARASCHIV[1], Aurelia DIACONU[1], Gheorghe COTEȚ[1], Ștefan NANU[1], Floriana Maria ȘTEFAN[2], Cristina BÎRSOGHE[1]

[1]Research Development Station for Plant Culture on Sands Dăbuleni,
217 Petre Baniță Street, Călăraşi, Dolj County, Romania
[2]National Institute for Research and Development for Potato and Sugar Beet Braşov,
2 Fundăturii Street, Braşov, Romania

Corresponding author email: alina22paraschiv@yahoo.com

Abstract

This study presents the research conducted at the Dăbuleni Research and Development Station for Plant Culture on Sands, which aimed at monitoring the physiological reactions of 11 potato genotypes, in order to determine their tolerance to the thermo-hydric stress characteristic of the sandy soil area in southern Oltenia. At an air temperature between 33.4-35.07°C and a very strong solar radiation, between 1800-2090 µmol/m²/s, the photosynthesis rate ranged between 6.08 µmol CO²/m²/s for the TS 12-1489-1576 genotype and 15.46 µmol CO²/m²/s for the 22-1941/8 genotype. Calculating the water use efficiency, as the ratio between the photosynthesis rate and transpiration (µmol / mmol), in all experimental variants supraunitary values were obtained, which demonstrates that the plants efficiently used the water lost through transpiration. From this point of view, the genotypes TS 09-1441-1525, TS 12-1489-1576 and TS 16-1527-1867 were highlighted. Between stomatal conductance and the physiological processes of photosynthesis and foliar transpiration, statistically significant correlations were established.

Key words*: photosynthesis, transpiration, climate, potato, sandy soils.*

INTRODUCTION

Potato, *Solanum tuberosum* (L.) is a plant species of the *Solanaceae* family and one of the top four widely cultivated crops in the world (Islam et al., 2024). The appeal of potatoes lies in their nutritional content and high production rate per unit time and area, making them particularly valuable in underdeveloped countries (Islam et al., 2022). In addition to being a staple food, potatoes are widely used in various industries to produce a diverse range of products. Furthermore, its wide range of applications, including food, alcohol, starch-based products, and raw materials, have made it quite popular in industrialized countries (Awasthi and Verma, 2017). Due to the vital importance of the potato, it is imperative to increase potato production. On the other hand, its production potential is often reduced due to its high susceptibility to numerous disease invasions, including various soil-borne pathogens and viruses (Awasthi and Verma, 2017; Chakrabarti et al., 2022). Such infestations increase the use of fungicides or insecticides for disease management, which reduces the profit margins of the growers (Islam et al., 2018). In addition to biotic pressures, abiotic factors such as drought, too low or too high temperatures, and salinity have caused challenges for producers during potato cultivation (Kikuchi et al., 2015). Therefore, both biotic and abiotic factors play a major role as stressors for potato cultivation. Potato tuber production is dramatically reduced under adverse environmental conditions. Due to abiotic stress, total production losses are expected to reach up to 32% by 2050. High temperatures and drought influence all biosynthetic processes in plants, but especially photosynthesis. In the case of plants exposed to moderate stress, which acclimatize, the synthesis of numerous proteins and enzymes occurs, allowing these plants to withstand the action of a more pronounced thermohydric stress. The optimal temperature for the photosynthesis process in potato plants is approximately 20°C according to Ku et al. (1977) and 30°C according to Hodson and Bryant (2012). The increase in temperature

determines the inhibition of the photosynthesis process and the stimulation of the respiration process, so that the intensity of the two processes can become equal, and the plants can no longer accumulate reserve substances (Burzo, 2014). Researchers and farmers alike are interested in measurements of photosynthesis intensity as a reaction of plants to applied technology, but also as a method of monitoring crop health or as a result of the effects of climate change.

At the Research Development Station for Plant Culture on Sands Dabuleni (RDSPCS) the research undertaken aimed at monitoring the physiological reactions of 11 potato genotypes, studied on different vegetation phenophases, in order to determine their tolerance to the thermo-hydric stress characteristic of the sandy soil area in southern Romania.

MATERIALS AND METHODS

Within the framework of the ADER 5.1.1 project Research on the impact of climate change on potato cultivation in traditional areas in order to reconfigure optimal cultivation areas in accordance with the EU "Green Deal" strategy and to identify and model the new potato ideotype with tolerance/resistance to climate change, an experimental field with biological material at different stages of the genetic improvement process, namely 11 potato genotypes (Figure 1), was established at RDSPCS Dabuleni in the spring of 2024.

Potato genotypes studied:

1. TS 09-1441-1525
2. TS 16-1515-1856
3. TS 16-1527-1867
4. TS 16-1526-1883
5. TS 12-1489-1576
6. Redsec (Control 1)
7. 1901/12
8. 1895/4
9. 1927/1
10. 22-1941/8
11. Ervant (Control 2)



Figure 1. Potato genotypes studied

In order to ensure early harvest, the tuber sprouting work was carried out one month before planting in the field. Thus, by planting the sprouted tubers, conditions were ensured for harvesting the tubers in June, thus avoiding the crop being subjected to the drought and heat periods in July-August characteristic of the southern area of Romania. Planting was carried out on February 22, 2024, semi-mechanized, consisting of opening the trenches mechanically, planting the sprouted tubers manually and then covering the trenches mechanically. The distance between the rows was 70 cm, and between the tubers in a row was 25 cm, achieving a planting density of about 57,000 plants/ha. The experiment was located in randomized blocks, in three repetitions. Since the natural fertility of the sandy soils in Oltenia is very low, before planting, it was fertilized with complex NPK fertilizer 15:15:15 at a dose of 100 kg a.s./ha, incorporated into the soil by a pass with a disc harrow, followed by a phased fertilization with ammonium nitrate 100 kg a.s./ha, approximately one week after the potatoes emerged.

From a physiological point of view, the processes of photosynthesis, transpiration and stomatal conductance were analyzed using the portable LC Pro SD system. With the help of this device, the gas exchange between plants and the environment was analyzed with the broad leaf chamber, during the period between the appearance of flower buds and the maximum flowering period of potato plants. LC Pro SD is an analysis system that can be easily used both in field and laboratory conditions, presenting the advantage that it has no negative effect on the analyzed plants, being non-destructive.

RESULTS AND DISCUSSIONS

Numerous specialists have followed the effects of thermal stress on horticultural plants. Thus, the limit temperature at which photosystem II in potato plants was irreversibly impaired is 38°C (Havaux, 1993). But plants that were exposed to a temperature of 40°C did not suffer obvious impairments in the activity of photosystem II, if they were acclimatized to temperatures between 30 and 35°C.

Tables 1-3 present the average results obtained at RDSPCS Dabuleni during the vegetation period between the appearance of the flower bud and the full flowering of the potato. The

intensity of the photosynthesis process of potato genotypes grown on sandy soils from Dabuleni (Table 1) varied depending on the genotype and their degree of adaptability to high thermal stress.

At an air temperature ranging between 33.4-35.07°C and a very strong solar radiation, ranging between 1800-2090 µmol/m²/s, the photosynthesis rate ranged between 6.08 µmol CO_2/m²/s for the *TS 12-1489-1576* genotype and 15.46 µmol CO_2/m²/s for the *22-1941/8* genotype.

As can be seen in Table 1, the results are presented in comparison with 2 controls, the *Redsec* and *Ervant* varieties. If we refer to the *Redsec* variety, a variety created at the Târgu Secuiesc Potato Research and Development Station, most of the genotypes analyzed recorded statistically significant negative differences, their photosynthetic capacity under heat stress conditions being much lower than that of the *Redsec* control variety. However, comparing the results obtained with the average values of the *Ervant* variety (control 2) created at the Research and Development National Institute for Potato and Sugar Beet Brașov, it was found that 8 of the genotypes differentiated positively, with values statistically assured as being distinctly significant and very significant. The genotypes *1927/1* and *22-1941/8* were noted to have increased tolerance to thermal stress.

Table 1. Photosynthesis rate in potatoes grown on sandy soils in Dăbuleni - 2024

Genotypes	Photosynthesis (µmol CO_2/m²/s)						
	Average value	Compared to *Redsec* (Control 1)			Compared to *Ervant* (Control 2)		
		Relative	Difference	Significance	Relative	Difference	Significance
TS 09-1441-1525	10.67	70.43	-4.48	ooo	140.95	3.10	**
TS 16-1515-1856	11.64	76.85	-3.51	oo	153.81	4.07	***
TS 16-1527-1867	10.96	72.34	-4.19	ooo	144.78	3.39	**
TS 16-1526-1883	12.08	79.76	-3.07	oo	159.62	4.51	***
TS 12-1489-1576	6.08	40.11	-9.07	ooo	80.27	-1.49	ns
Redsec (control 1)	15.15	100.00	0.00	Control 1	200.13	7.58	***
1901/12	9.34	61.65	-5.81	ooo	123.38	1.77	ns
1895/4	11.55	76.24	-3.60	oo	152.58	3.98	**
1927/1	15.19	100.26	0.04	ns	200.66	7.62	***
22-1941/8	15.46	102.02	0.31	ns	204.18	7.89	***
Ervant (control 2)	7.57	49.97	-7.58	ooo	100.00	0.00	Control 2
DL		5% = 2.21		1% = 3.00		0.01% = 4.07	

The transpiration process, dependent on both the temperature level and the water content of the soil and the plant, took place with an intensity directly proportional to the photosynthesis rate (Table 2). The average values of this physiological process ranged between 0.74 mmol H_2O/m²/s for the *TS 12-1489-1576* genotype and 3.96 mmol H_2O/m²/s for the *22-1941/8* genotype. The genotypes *1927/1* and *22-1941/8* transpired the most intensely, for which positive, statistically significant differences were recorded, both in comparison with the *Redsec* variety and the *Ervant* variety. Calculating water use efficiency, as the ratio between the rate of gross photosynthesis and transpiration (µmol / mmol), in all experimental variants supra-unitary values were obtained, which demonstrates that the plants efficiently used the water lost through transpiration. From this point of view, the genotypes TS 09-1441-1525, TS 12-1489-1576 and TS 16-1527-1867 were highlighted.

Table 2. Transpiration rate of potatoes grown on sandy soils in Dăbuleni - 2024

Genotypes	Transpiration (mmol $H_2O/m^2/s$)						
	Average value	Compared to *Redsec* (Control 1)			Compared to *Ervant* (Control 2)		
		Relative	Difference	Significance	Relative	Difference	Significance
TS 09-1441-1525	1.12	36.12	-1.99	ooo	68.92	-0.51	ooo
TS 16-1515-1856	1.67	53.80	-1.44	ooo	102.66	0.04	ns
TS 16-1527-1867	1.54	49.52	-1.57	ooo	94.48	-0.09	ns
TS 16-1526-1883	2.24	72.03	-0.87	ooo	137.42	0.61	***
TS 12-1489-1576	0.74	23.90	-2.37	ooo	45.60	-0.89	ooo
Redsec (control 1)	3.11	100.00	0.00	Control 1	190.80	1.48	***
1901/12	1.55	49.84	-1.56	ooo	95.09	-0.08	ns
1895/4	2.02	65.06	-1.09	ooo	124.13	0.39	***
1927/1	3.33	107.07	0.22	*	204.29	1.70	***
22-1941/8	3.96	127.44	0.85	***	243.15	2.33	***
Ervant (control 2)	1.63	52.41	-1.48	ooo	100.00	0.00	Control 2
DL		5% = 0.17		1% = 0.24		0.01% = 0.32	

Table 3. Stomatal conductance in potatoes grown on sandy soils from Dăbuleni - 2024

Genotypes	Stomatal conductance ($mol/m^2/s$)						
	Average value	Compared to Redsec (control 1)			Compared to Ervant (control 2)		
		Relative	Difference	Significance	Relative	Difference	Significance
TS 09-1441-1525	0.063	52.78	-0.057	ooo	134.75	0.016	***
TS 16-1515-1856	0.080	66.67	-0.040	ooo	170.21	0.033	***
TS 16-1527-1867	0.060	50.00	-0.060	ooo	127.66	0.013	***
TS 16-1526-1883	0.083	69.44	-0.037	ooo	177.30	0.036	***
TS 12-1489-1576	0.020	16.67	-0.100	ooo	42.55	-0.027	ooo
Redsec (control 1)	0.120	100.00	0.000	Control 1	255.32	0.073	***
1901/12	0.050	41.67	-0.070	ooo	106.38	0.003	ns
1895/4	0.070	58.33	-0.050	ooo	148.94	0.023	***
1927/1	0.127	105.56	0.007	*	269.50	0.080	***
22-1941/8	0.167	138.89	0.047	***	354.61	0.120	***
Ervant (control 2)	0.047	38.89	-0.073	ooo	99.29	0.000	Control 2
DL		5% = 0.007		1% = 0.009		0.01% = 0.013	

A particularly important role in determining the intensity of physiological processes in leaves is played by stomata, the degree of their opening being in close correlation with environmental factors (especially light and temperature). The higher the values of stomatal conductance, the more intense the gas exchange between plants and the environment. In periods of very high temperatures and extremely strong solar radiation, plants defend themselves by closing the stomatal ostioles, which also determines the reduction of the intensity of physiological activity in leaves. Under conditions of thermal stress, stomatal conductance recorded average values ranging between 0.020 $mol/m^2/s$ at *TS 12-1489-1576* and 0.167 $mol/m^2/s$ at *22-1941/8* genotype (Table 3).

Statistically significant correlations were established between stomatal conductance and the physiological processes of photosynthesis and foliar transpiration (Figure 2). The higher the stomatal conductance values, the more the two physiological processes analyzed were intensified.

The increase in temperature is also accompanied by an increase in the intensity of solar radiation, which can exceed the saturation degree of the photoreceptor antennas. Under these conditions, the activity of the oxygen-producing complex is affected and the photoreceptor activity of photosystem II decreases, which determines the inhibition of photosynthesis and the generation of the photoinhibition process. This can be seen in Figure 3, which illustrates the negative correlations established between the solar radiation active in photosynthesis and the two processes analyzed. Both photosynthesis and foliar transpiration intensified up to solar radiation values of 1900 µmol/m²/s, after which a reduction in the intensity of physiological activity is observed, with potato plants activating their natural defense mechanisms against thermal stress (hydropassive closure of stomata and photoinhibition).

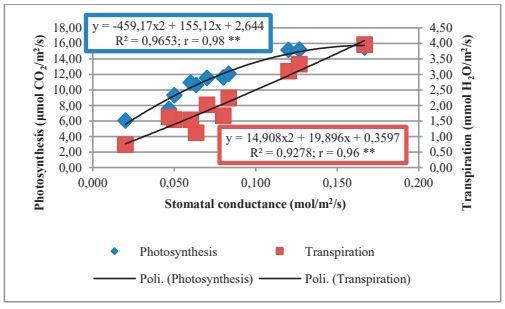


Figure 2. Correlations between stomatal conductance, photosynthesis and transpiration in potatoes

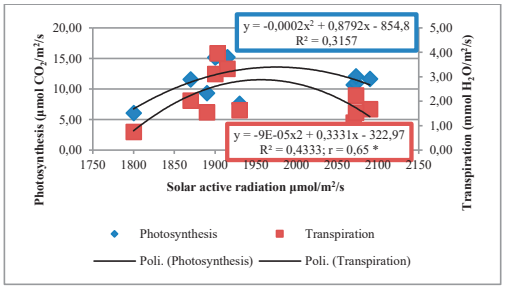


Figure 3. Correlations between solar radiation, photosynthesis and transpiration in potatoes

CONCLUSIONS

To determine the suitability of certain potato genotypes for cultivation on sandy soils, it is necessary to study their behavior over several years.

The partial results obtained highlighted differences depending on the genetic potential and tolerance of each genotype to thermo-hydric stress, from this point of view the genotypes *1927/1* and *22-1941/8* stand out.

Climate change will probably further aggravate the productivity of potatoes by intensifying the exposure of potato plants to these stress conditions. Therefore, there is an urgent need to adapt to the new challenges generated by climate change, improving the adaptability of potato plants to environmental stress and obtaining new varieties, tolerant to heat, drought, phytopathogenic stresses, being the most important and challenging objectives.

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ENZYMATIC AND ANTIOXIDANT ACTIVITIES OF SEVERAL EDIBLE MUSHROOMS SPECIES

Rodica SOARE[1], Maria DINU[2], Cristina BĂBEANU[3]

[1]University of Craiova, Faculty of Agriculture, 19 Libertatii Street, Craiova, Dolj, Romania
[2]University of Craiova, Faculty of Horticulture, 13 A.I. Cuza Street, Craiova, Dolj, Romania
[3]University of Craiova, Faculty of Science, 13 A.I. Cuza Street, Craiova, Dolj, Romania

Corresponding author email: dinumariana@hotmail.com

Abstract

Edible mushrooms are widely consumed for their nutritional value, bioactive compound content, and good taste. The objective of this study was to evaluate and compare the enzymatic and the antioxidant activities of six species commonly marketed in Romania (Pleurotus ostreatus, Agaricus bisporus white, Agaricus bisporus brown, Lentinula edodes (Shiitake), Shimeji white and Shimeji brown. The enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and soluble peroxidase (POX) as well as the antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS (2, 2'- azino -bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation were determined by colorimetric methods. The studied mushroom species have appreciable antioxidant enzymatic activities with an important role in human health. Pleurotus ostreatus is distinguished as having the highest antioxidant activity and high enzymatic activity. The results of this study recommend introducing the investigated mushrooms in diet as a source of exogenous antioxidants.

Key words*: antioxidant, enzymes activity, mushrooms, polyphenols.*

INTRODUCTION

Recently, the population's attention has been focused on diet, a key factor affecting health and the incidence of many diseases. Nutritional research has highlighted functional foods rich in bioactive compounds whose consumption is associated with well-being and healthy living. Mushrooms are widely consumed and appreciated as functional foods due to their organoleptic, nutritional and medicinal properties (Fogarasi et al., 2024; Yadav & Negi, 2021). Mushrooms are low in calories with low fat content and are rich in polyunsaturated fatty acids which are mainly in the form of linoleic, α-linolenic and oleic acids (Reiss et al., 2012; Fogarasi et al., 2020). Mushroom proteins, which constitute 19-35% of dry weight, contain an optimal qualitative and quantitative spectrum of the full range of essential amino acids, making them a good alternative to animal proteins (Ayimbila & Keawsompong, 2023).

Some proteins and peptides produced by mushrooms such as lectins, fungal immunomodulatory proteins, ribosome-inactivating proteins, antimicrobial proteins, ribonucleases, and laccases present important biological properties (Xu et al., 2011).

Mushrooms are a good source of minerals such as potassium, phosphorus, sodium, calcium, magnesium, and essential microelements required for human physiological functions such as copper, zinc, iron, molybdenum and selenium with antioxidant and anti-inflammatory properties (Senila et al., 2024). Mushrooms are a good source of vitamins, especially B vitamins, folic acid and ergosterol which can be easily converted into vitamin D2. Ultraviolet light exposure from sunlight can promote vitamin D production in mushrooms. Sun et al. (2022) report that ergosterol level varies between 2290 and 6200 μg/g in edible mushrooms *Agaricus bisporus* and *Pleurotus ostreatus* and after UV exposure vitamin D content range between 25.9-742 μg/g according to the UV irradiation method (Sun et al., 2022.)

Mushrooms are rich in dietary fiber (Flores et al., 2024) as well as polysaccharides, heteroglucans, peptidoglucans, and proteoglucans. Mushrooms are a complex source of numerous bioactive molecules and health promoting compounds that include: polyphenolic compounds, terpenes, alkaloids,

sterols, polysaccharides (Flores et al., 2024), peptides, proteins, lectins (Abdelshafy et al., 2022; Hamza et al., 2023; Yadav & Negi, 2021, Fogarasi et al., 2024).

Mushrooms contain numerous enzymes such as laccase, lipase, cellulase, peroxidase, amylase, xylanase, protease and pectinase (Sami et al., 2023; Slawinska and Kalbarczyk, 2011). Many of the enzymes extracted from mushrooms are used in the food industry in food processing or to increase the nutritional quality of foods.

In addition to their nutritional qualities, some edible mushrooms have multiple therapeutic properties, being rich in phytocompounds with pharmacological activity (Assemie & Abaya, 2022). Mushrooms are used to prevent and combat many diseases, having antioxidant, antitumor, antimicrobial, antiviral, antiobesity, antidiabetic, antinflammatory, detoxifying, immunemodulatory, hypoglycemic, cholesterol lowering, hepatoprotective, and analgesic properties (Hamza et al., 2024; Venturella et al., 2021). Many studies report positive effects as potential chemopreventive agents in various types of cancer (Ajith et al., 2007). Bioactive compounds from mushrooms have inhibited the growth of cancer cells and induced apoptosis (Sreedharan et al., 2025). Mushrooms contain phytocompounds with anti-inflammatory properties, managing with positive effects conditions such as arthritis, inflammatory liver and intestinal diseases (Chopra et al., 2021). The variation in phytochemicals content in mushrooms is dependent on many factors: genotype, stage of harvesting, growing conditions, substrate, storage conditions (Perez Montes et al., 2021).

Researches were carried out focused on the identification and selection of species that ensure a high nutritional value for the consumer. In this context, the purpose of this study was to evaluate and compare the phenolic compounds content, the enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and soluble peroxidase (POX) and the antioxidant activity of six species of cultivated mushrooms commonly marketed in Romania: *Pleurotus ostreatus, Agaricus bisporus* white; *Agaricus bisporus* brown, *Lentinula edodes (Shiitake), Shimeji* white and *Shimeji* brown.

MATERIALS AND METHODS

The biological material was represented by fruiting bodies of six cultivated mushroom species commonly marketed in Romania: *Pleurotus ostreatus, Agaricus bisporus* white, *Agaricus bisporus* brown, *Lentinula edodes (Shiitake, Shimeji* white and S*himeji* brown. The mushrooms were purchased from the local supermarket.

Reagents: Methanol used for the extraction was from Sigma-Aldrich. Gallic acid, ascorbic acid, 1.1-diphenyl-2-picrylhydrazyl, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 6-hydroxy-2,5,7,8-tetramethylchromon 2-carboxylic acid (Trolox), nitroblue tetrazolium chloride (NBT), were purchased from Sigma (Sigma-Aldrich, Sternheim, Germany). Folin-Ciocalteu reagent and guaiacol were purchased from Merck, (Darmstadt, Germany). All the other used chemicals were of analytical grade.

Sample preparation

For antioxidant enzymes extraction, fresh and mature whole fruiting bodies mushroom were homogenised with 0,1M phosphate buffer (pH 7.5) containing 0,01M EDTA. The homogenates were centrifuged for 5 minutes at 6000 g in a Hettich Rotina 380 centrifuge (Tuttlingen, Germany) and the supernatants were used for enzyme assays.

Methanolic extract: For the determination of antioxidant activity and total phenolic content the samples were extracted with 80% aqueous methanol (1:10 w:v) by sonicating for 60 min in a sonicate bath Fungilab (Madrid, Spain) equipped with a digital timer and a temperature controller at 24°C. The resulting slurries were centrifuged at 10000 g for 10 minutes and the supernatants were analyzed.

Biochemical analysis

The Superoxide dismutase (EC 1.15.1.1) activity (SOD) was assayed by measuring ability of sample extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Superoxide dismutase activity IC50 % was expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of NBT (Soare et al., 2017).

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in absorbance at 470 nm (A470) due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 and their activity was expressed as ΔA/min/1g f.w. (Soare et al., 2017).

Catalase (E.C.1.11.1.6) activity (CAT) was assayed through the spectrophotometric method at 240 nm by recording the decrease of absorbance due to H_2O_2 consumption (Soare et al., 2017). The results were expressed as μM H_2O_2 /min/g f.w at 25°C.

The total phenolics content (TPC) was determined colorimetric by using the Folin-Ciocalteu method based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. 2 mL Folin-Ciocalteu's phenol reagent (1:10) and 1.5 mL 7.5 % w/v Na_2CO_3 were added to 0.5 mL methanolic sample extract. The mixture was allowed to stand at room temperature in the dark for 60 min and then the absorbance was recorded at 765 nm (Dinu et al., 2018). The TPC was calculated using a standard curve prepared using gallic acid and expressed as mg GAE/1 g fw.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The capacity of methanolic sample extract to reduce the radical 2,2-diphenyl-1-picrylhydrazyl has been evaluated colorimetrically at 517 nm (Dinu et al., 2018). The results values of percentage of DPPH free radical scavenging were compared with those obtained from two standard curves (Trolox and ascorbic acid). Antioxidant capacity values were expressed as μM TE/g fw and μM AsA/g fw respectively.

ABTS (2, 2'- azino -bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity was measured colorimetric at 734 nm using Trolox or ascorbic acid as standard. The final results were expressed as μM TE/g fw and μM AsA/g fw (Soare et al., 2017).

The spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software. All determinations were performed in triplicate.

Statistical Analysis

The data obtained were analyzed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), and the means were compared using Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

In this paper, the enzymatic activity of catalase, peroxidase and superoxide dismutase, enzymes that are the primary enzymatic defenses against free radicals, was determined. SOD, CAT and POX are important active free-radical scavenging enzymes. SOD is an enzyme localized in cytosol, chloroplast, mitochondria and peroxisomes and catalyses the dismutation of the superoxide anion into oxygen and hydrogen peroxide and CAT was responsible for eliminating H_2O_2. Catalase, absent in chloroplast is localized in mitochondria and peroxizomes. This enzyme catalyses the reduction of hydrogen peroxide to H_2O and molecular oxygen. Catalase and peroxidases are among the most studied enzymes in fruits and vegetables. Catalase and peroxidase have different affinities for reactive oxygen species and appear to have different cellular roles in hydrogen peroxide scavenging. Catalase does not need a reductant to scavenge H_2O_2 whereas peroxidase needs a reductant. Catalase has a lower affinity for hydrogen peroxide (mM range) than peroxidase (μM range) (Cruz de Carvahlo, 2008). Peroxidase may function as a fine regulator of intracellular reactive oxygen species whereas catalase might function as a bulk remover of excess reactive oxygen species production under stress conditions (Cruz de Carvahlo, 2008). Hydroxyl radical and hydrogen peroxide are the most toxic species because they can nonspecifically oxidize all classes of biological macromolecules and their elimination from the cell through the action of these enzymes is very important.

In this paper the obtained results show that the activity of antioxidant enzymes varies with the investigated mushroom species (Table 1).

The results obtained for superoxide dismutase activity, expressed as IC 50% (mg), vary between 0.882 mg (*Agaricus bisporus* brown) and 3.33 mg (*Shimeji* brown) as follows: *Shimeji* brown > *Lentinula edodes* > *Shimeji* white > *Agaricus bisporus* white > *Pleurotus ostreatus* > *Agaricus bisporus* brown (Table 1).

In the case of catalase, the enzymatic activity ranges from 0.949 mM H_2O_2/min/g fw (*Shimeji*) white) to 3.248 mM H_2O_2/min/g fw (*Pleurotus ostreatus*).

Table 1. Enzymatic activity of investigated mushrooms

	SOD IC50 (mg)	CAT mM H_2O_2/min /g fw	POX ΔA/min/ 1g f.w
Pleurotus ostreatus	0.926d	3.248a	0.98a
Agaricus bisporus white	0,95d	1.583c	0.84b
Agaricus bisporus brown	0.882d	2.317b	0.78b
Lentinula edodes (Shiitake)	2.5b	2.415b	0.88ab
Shimeji white	1.612c	0.949d	0.8b
Shimeji brown	3.33a	1.418c	0.75b
LSD 5%	0.182	0.299	0.136

Mean values with different letters significantly differ, and statistical significance was set at $p < 0.05$.

The literature is poor in data regarding catalase and peroxidase from mushrooms. In a study that investigate several mushrooms from Dambovita, Romania, in terms of enzymatic and non-enzymatic antioxidants, *Agaricus bisporus* brown was shown to have the highest catalase and peroxidase activity among cultivated mushrooms (Radulescu et al., 2019). Susmitha et al. (2013) show that mushrooms are a good source for the extraction and purification of catalase which can be used efficiently as a therapeutic treatment **(**Susmitha et al., 2013**)**. The medicinal properties of mushroom catalases have been attributed to inhibition of platelet aggregation reduction of blood cholesterol and glucose concentrations, prevention or alleviation of heart disease also prevention or alleviation of infections caused by bacterial, viral, fungal and parasitic pathogens (Susmitha et al., 2013)

The results obtained for peroxidase activity are shown in Table 1. In the case of peroxidase, the enzymatic activity ranges from 0.75 ΔA/min/1 g f.w (*Shimeji* brown) to 0.98 ΔA/min/1 g f.w (*Pleurotus ostreatus*). Peroxidase (POX) is an heme iron protein that is mostly located in the cell wall. This enzyme has been recognized to be involved in the control of development, lignification, pathogen defense and the catabolism of growth regulators (Van Huystee, 2003). Among the investigated mushroom species *Pleurotus ostreatus* stands out with the highest antioxidant enzymes activities while Shimeji brown has the lowest POX and SOD activity. The lowest CAT activity is found in Shimeji white.

The content of total phenolic compounds (TPC) varies depending on the analyzed mushroom species (Table 2). TPC varies between 0.6085 mg GAE/1 g fw and 1.538 mg GAE/1 g fw in the order: *Pleurotus ostreatus*>*Agaricus bisporus* brown> *Agaricus bisporus* white> *Shimeji* brown > *Lentinula edodes* > *Shimeji* white. The highest TPC was determined in the *Pleurotus ostreatus* being 2.53 times higher than in *Shimeji* white mushrooms.

The data reported in the literature for the content of phenolic compounds vary because the content of bioactive compounds in mushrooms may vary depending on many factors and the extraction method (Yim et al., 2009; Perez Montes et al., 2021).

The results obtained in our study are higher than those presented in other papers investigating the same species: in five selected wild edible mushrooms from Romania TPC varies between 408.57 mg GAE/100 g DW and 806.58 mg GAE/100 g DW (Fogarasi et al., 2020); in eight types of edible mushrooms TPC varies between 1 and 6 mg of phenolics per gram of dried mushroom, depending on the species (Palacios et al., 2011). In 43 commonly consumed mushrooms in China TPC varies between 0.8 mg GAE/1 g dw and 11.93 mg GAE/1 g dw (Islam et al. 2016).

For *Agaricus bisporus*, TPC was found to be 4020 mg GAE/kg DW (Keles et al., 2011), and 2.04 mg GAE/g Dw (Robaszkiewicz, 2010); and for *Pleurotus ostreatus* 2686 mg GAE/kg DW (Keles et al., 2011) and 1.44 mg GAE/g DW (Robaszkiewicz, 2010);

Other authors present similar results to our study: TPC in the range of 4.2 to 10.6mgGAE/g dw (Dubost et al., 2007); TPC concentration for the five commercial mushrooms ranged from 5.66 to 13.16 mg GAE/g dm. (Bach et al., 2019) but in other studies the reported data are higher than our results (Radulescu et al., 2019, Boonsong et al, 2016). In a study exploring sixteen of the most popular edible wild mushroom species as potential sources of antioxidants, the highest total polyphenol content, exceeding 100 mg/100 g fresh mass, was found in five mushrooms (Witkowska et al., 2011).

Table 2. Total phenolic content and antioxidant activity in investigated mushrooms species

Mushrooms species	TPC (mg GAE/ g fw)	Antiox. activity ABTS (µM TE/ g fw)	Antiox. activity ABTS (µM AsA E/g fw)	Antiox. activity DPPH (µM TE/g fw))	Antiox. activity DPPH (µM AsA E/ g fw)
Pleurotus ostreatus	1.538 a	1.995 a	1.815 a	2.711 a	1.961 a
Agaricus bisporus white	0.984 c	1.113 d	0.908 d	2.173 b	1.612 b
Agaricus bisporus brown	1.108 b	1.525 b	1.336 b	1,779 d	1.350 c
Lentinula edodes (Shiitake)	0.638 d	0.928 e	0.720 c	0.828 f	0.718 e
Shimeji white	0.609 d	1.121 d	0.927 d	0.881 f	0.754 e
S*himeji* brown	0.673 d	1.216 c	1.022 c	1.270 c	1.012 d
LSD 5%	0.099	0.093	0.066	0.116	0.113

Mean values with different superscript letters significantly differ, and statistical significance was set at $p < 0.05$.

Numerous studies show the presence of phenolic compounds in the composition of mushrooms, the most common being: gallic acid, gallic, protocatechuic, p-hydroxybenzoic, pcoumaric, cinnamic, salicylic, syringic, ferulic, chlorogenic and caffeic acids and a wide spectrum of flavonoid compounds (Yim et al., 2009; Islam et al., 2016; Reis et al., 2012). It has been proven that the phenolic compounds from mushrooms present several biological activities such as antioxidant, antitumor, antimicrobial, hypoglycemic, and anti-inflammatory activities, explaining many of the beneficial effects of mushrooms. The antioxidant character of phenols is determined by their redox properties. Polyphenols protect the cell against oxidative damage caused by free radicals. It is known that free radicals in excess are involved in the occurrence and development of many diseases including cancer, diabetes, cataracts, rheumatoid arthritis, neurodegenerative diseases, cardiovascular problems and degenerative processes associated with aging (Cruz de Carvalho, 2008).

In this study antioxidant activity of the investigated mushroom species was determined by the ability of extracts to scavenge the DPPH and ABTS cation radical which are the most accepted and used methods of evaluating antioxidant activity. The results were calculated using two standards: Trolox and ascobic acid.

The values of DPPH radical scavenging activity ranged from 0.8278 µM TE/g fw to 2.711 µM TE/g fw in terms of Trolox equivalents and from 0.7184 µM AsA/g fw to 1.961 µM AsA/g fw in terms of Ascorbic acids equivalents in order: *Lentinula edodes (Shiitake)*< *Shimeji* white < *Shimeji* brown < *Agaricus bisporus* brown < *Agaricus bisporus* white< *Pleurotus ostreatus* (Table 2). The antioxidant activity was also measured and compared for free radical scavenging activities against ABTS radical cation (Table 2). All results showed significant ABTS radical cation scavenging activity which ranged from 0.9279 µM TE/g fw to 1.9954 µM TE/g fw in terms of Trolox equivalents and from 0.72 µM AsA/g fw to 1.815 µM AsA/g fw in terms of Ascorbic acids equivalents. *Agaricus bisporus* white has ABTS cation radical scavenging activity value (1.1128 µM TE/g fw and 0.908 µM AsAE/g fw) close to the values determined for Shimeji white (1.1205 µM TE/g fw; 0.927 µM AsAE/g fw) while DPPH radical scavenging activity (2.173 µM TE/g fw; 1.6118 µM AsAE/g fw) is 2.46 times higher than the value for Shimeji white in terms of Trolox equivalent (0.8814 µM TE/g fw) and 2.13 times higher in terms of Ascorbic acid equivalent (0.7539 µM AsAE/g fw). The different results determined by the two methods are explained by the kinetics of the reactions between phenols and the types of free radicals, the spectrum and concentration range of phenols (Henriquez et al., 2004).

Numerous bioactive compounds in the composition of mushrooms are responsible for their antioxidant properties (Fogarasi et al., 2024, Gan et al., 2013). The concentration of these compounds varies with different factors and we find the same influence on antioxidant activity. Mushrooms can be used as an exogenous source of antioxidants to help the body defend against the harmful effects of excess free radicals.

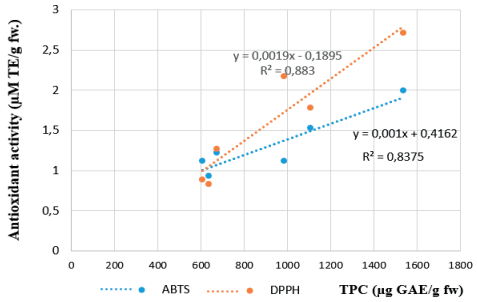


Figure 1. Corelation between antioxidant activity expressed as Trolox equivalent and total phenolic content

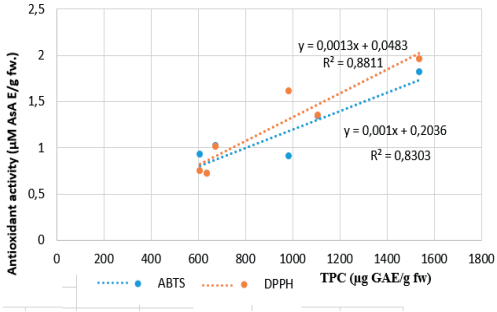


Figure 2. Corelation between antioxidant activity expressed as Ascorbic acid equivalent and total phenolic content

A significant positive correlation was observed between DPPH or ABTS radicals scavenging capacity and TPC suggesting that the antioxidant activity is due to the content of phenolic compounds (Figure 1 and Figure 2). Although mushrooms also have antioxidant enzymatic activity, this probably does not represent a major contribution to the antioxidant activity. The positive correlation between antioxidant activity and TPC was also reported in other studies (Gan et al., 2013; Fogarasi et al., 2020; Puttaraju et al., 2006; Dubost et al., 2007) and for other plant species (Dinu et al., 2018; Dinu et al., 2021).

CONCLUSIONS

The obtained results show that studied biochemical indices vary depending on the analyzed species, the studied mushrooms having high phenolic content and high antioxidant activity.

Among the investigated mushroom species *Pleurotus ostreatus* stands out with the highest antioxidant enzymes activities and antioxidant activity. *Lentinula edodes* (Shiitake) has the lowest antioxidant activity and a low phenolic content. The obtained results show that Shimeji brown has the lowest POX and SOD activity while the lowest CAT activity is found in Shimeji white.

Despite the fact that the enzymatic activity analyzed is not very high, mushrooms can be recommended as beneficial in protecting against oxidative stress.

The studied mushrooms can be used as functional foods being a rich source of bioactive compounds with high nutritional value and health promoting properties.

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THE INFLUENCE OF SOIL TYPES ON SEEDS GERMINATION AND PLANTS HEALTH TO DIFFERENT SPECIES OF VEGETABLES

Gabriela ȘOVĂREL[1], Marcel COSTACHE[1], Ana-Emilia CENUȘĂ[1], Simona HOGEA[1], Marius VELEA[2]

[1]Research and Development Institute for Vegetable and Flower Growing Vidra, 22 Calea București Street, 077185, Vidra, Ilfov, Romania
[2]Holland Farming Agro SRL, 74 Drumul Osiei Street, 062395, Bucharest, Romania

Corresponding author email: gabriela_sovarel@yahoo.com

Abstract

Vegetable yield is significantly influenced by the pedoclimatic conditions in the growing areas, by the applied technology and by the level of soil infection with different pathogens. We worked with 7 soil samples from Cluj County and 7 samples from Arad County. The soil samples were analyzed in terms of infection levels with various soil pathogens that cause seed rot and `damping-off`. The presence of pathogens was determined by the `trap plants` method. The experiment was performed in laboratory conditions, at an average temperature of 20.6-26.0°C and average relative humidity of 34.5-46.4%. In the soil samples it was determined the presence of soilborne pathogens Pythium debaryanum, Phytophthora parasitica and Rhizoctonia solani which cause seed rot and `damping-off`. Soil samples from Cluj County presented the largest number of `fallen` cabbage plants. Arad County showed the highest number of `fallen` tomato plants, 38 `fallen` cucumber plants.

Key words*: pathogens, nutrients, damping-off, Pythium debaryanum, Phytophthora parasitica, Rhizoctonia solani.*

INTRODUCTION

Vegetable yield is significantly influenced by the pedoclimatic conditions in the growing areas, by the applied technology, and by the level of soil infection with different pathogens. Soilborne pathogens *Pythium debaryanum, Phytophthora parasitica* and *Rhizoctonia solani* can cause seed rot and damping-off in most vegetable species where crops are established by directly sowing. The attack of the fungus *Pythium debaryanum* on vegetable seedlings has an acute character, a tomato plant can be destroyed, depending on temperature and humidity, in 12-24 hours, and in the greenhouses for seedlings production, in a few days, they can perish most plants (Baicu et al., 1996). *Phytophthora parasitica* manifests itself in temperature conditions between 20 and 25°C and in the presence of a film of water (Costache et al., 2018; Șovărel et al., 2020). *Rhizoctonia solani* is a polyphagous pathogen (over 25 known hosts), able to survive on plant debris from various plant species. Contamination is possible also through horticultural substrates (Blancard, 1997). The present researches aimed to establish the infectious potential of 14 soil samples from Cluj and Arad counties collected from the field and protected areas. Knowing the role of nutrients in plant life and their influence on harvest quality is of particular importance, because it underpins the measures that are required to ensure optimal plant growth and development and the production of superior quality plant products (Budoi, 2000). Tomato plants require at least twelve nutrients, also called “essential elements”, for normal growth and reproduction. These are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and molybdenum (Mo) (Sainju et al., 2003).

MATERIALS AND METHODS

To detect the presence of soil pathogens, in the soil samples from Cluj and Arad counties, the "trap plants" method was used. At the samples from Cluj county we worked with cauliflower (49S, 50S), cabbage (66S, 67S, 68S, 70S), and celery (69S) and at the samples from Arad county with pepper (90S, 92S), tomatoes (91S, 93S, 94S, 96S) and cucumbers (95S). Plastic trays measuring 25 cm long, 20 cm wide and 5

cm high in which the soil samples were placed were used. On the same day, in the trays thus prepared, 100 seeds (10 rows of 10 seeds) of cauliflower (Herfstreusen variety), cabbage (Buzău variety), celery (Dolvi variety), peppers (Asteroid 204 variety), tomatoes (Pontica 102 variety) and cucumbers (Cornichon de Paris variety) were sown. After sowing the seeds were covered with 0.5 cm of soil (from the same soil sample) and watered with the same amount of water. Next, every 2 days, the dynamics of the emergence of these 6 species of vegetables (cauliflower, cabbage, celery, peppers, tomatoes, cucumbers) and the appearance and evolution of the attack of the mentioned soil pathogens were followed (frequency of rotten seeds, frequency [%] "damping-off". During the experiment, the temperature and atmospheric humidity in the room in which it was placed were permanently recorded using a thermohygrometer type EMC DT - 172. In the experimental period (08.02.2021-02.03.2021) the minimum temperature was 19.8-24.1°C, the average temperature was 20.6-26.0°C, and the maximum temperature was 21.5-33.3°C. The minimum relative humidity varied between 23.5 and 42.5%, the average relative humidity between 34.5 and 46.4%, and the maximum relative humidity between 38.2 and 49.0%.

RESULTS AND DISCUSSIONS

Table 1 shows the samples code, the county and the location where the samples were taken, the origin of the sample, and the previous culture.

Table 1. The provenance of the analyzed soil samples

Nr. Crt.	Samples code/ county	Location	Provenance of sample	Previous crops
0	1	2	3	4
1.	49 S/Cluj	Apahida	Field	cauliflower
2.	50 S/Cluj	Sânnicoară	Field	cauliflower
3.	66 S/Cluj	Câmpia Turzii	Field	cauliflower
4.	67 S/Cluj	Câmpia Turzii	Field	cabbage
5.	68 S/Cluj	Viișoara	Field	cabbage
6.	69 S/Cluj	Viișoara	Field	celery
7.	70 S/Cluj	Viișoara	Field	cabbage
8.	90 S/Arad	Macea	Field	bell pepper
9.	91 S/Arad	Apateu	high plastic tunnels	tomato, cucumbers
10.	92 S/Arad	Nădlag	Field	hot pepper
11.	93 S/Arad	Apateu	high plastic tunnels	tomato, bell pepper
12.	94 S/Arad	Apateu	high plastic tunnels	tomato
13.	95 S/Arad	Olari	high plastic tunnels	cucumbers
14.	96 S/Arad	Apateu	high plastic tunnels	Tomato

Samples from Cluj County (Table 2)

In general, the soil is weakly alkaline, well supplied with organic matter, but which in some cases has relatively high concentrations of sodium; it's about solonchak soils. It recommended that the soil will be amended with gypsum - CaSO4.2H2O, phosphogypsum (residual material of phosphate fertilizer plants), calcium chloride - CaCl2.6H2O, Al sulphate, Fe sulphate, native sulfur, or lignite powder.

- pH: soil reaction varies between neutral (7.06) and weakly alkaline. Under these conditions, the more alkaline the pH, the lower the mobility and availability of nutrients for plants: phosphorus, manganese, boron, zinc, and relatively magnesium, iron, and copper. The most affected is phosphorus, which influences the growth of the root system, and together with boron the development of plants. Foliar fertilization with fertilizers rich in assimilable phosphorus and boron is recommended.
- ammoniacal nitrogen: it is relatively good; at the seedling stage, when the energetic state of the plants is precarious and in conditions of deficient solar radiation, however, higher values are recommended, but not more than 1/10 of the nitric nitrogen level.
- potassium: varies from low (sample 2-50S) to very low. Where the values are very low-low, root fertilizers with potassium sulfate are needed, which will also correct the pH.
- calcium: normally should be at least equal to water-soluble potassium, if not even higher by 50%. Samples 1 (49S) and 5 (68S) can lead to calcium deficiency, in which case the resistance to pathogens (fungi and bacteria) decrease.
- magnesium: the optimal values, depending on the cultivated species, vary between 1/2 and 1/4 of the potassium values. Where the balance is in favor of magnesium, potassium deficiency may occur (and vice versa). This is the case for samples 3 (66S), 4 (67S), 5 (68S) and 7 (70S). Due to its role in energy metabolism, protein, and especially nucleic acids, it increases the resistance of plants to pathogens (fungi and

bacteria). Its lack reduces the seed germination faculty.
- nitrites + nitrates: except for sample 3 (66S), the values are very low. Additional fertilization (practically every watering) with ammonium nitrate and alternating (one in three) with urea is required. The importance of nitrogen, especially nitric, on plant growth is known. Its lack increases their sensitivity to the attack of harmful organisms.
- chlorides: they present acceptable values.
- sulphates: except for the values corresponding to samples 2 (50S) and 4 (67S), the other samples are acceptable.
- phosphorus: all samples, except sample 2 (50S), reflect phosphorus-poor soils for field-grown vegetables.
Phosphorus deficiency increases the sensitivity of plants to: *Alternaria, Botrytis, Phytophthora, Fusarium* and *Erwinia*.
High phosphorus values facilitate the absorption of nitric nitrogen (sample 2-50S, whose concentration of nitric nitrogen is very low).
Basic fertilization with superphosphate or complex 22.22.0 or 16.48.0 or in vegetation with MAP (monoammoniacal phosphate) is required.

Table 2. The content in the main nutrients of soil samples from Cluj county

County	Samples no. and code	Determinations performed*										
		pH	N-NH_4	K mg/kg	Na mg/kg	Ca mg/kg	Mg mg/kg	N mg/kg	Cl^- mg/kg	SO_4^{2} mg/kg	P mg/kg	Humus
Cluj	1 (49S)	8.19	<1.25	<1.25	<1.25	41.1	<1.25	3.9	3.5	52.1	30.3	2.34
	2 (50S)	8.05	1.9	87.2	<1.25	114.0	35.2	9.3	8.0	239.6	262.8	4.47
	3 (66S)	7.06	<1.25	7.0	6.7	59.6	13.3	23.4	17.4	55.5	43.8	4.95
	4 (67S)	8.11	<1.25	4.0	36.3	146.2	9.3	7.7	26.7	173.6	11.0	2.14
	5 (68S)	7.86	<1.25	7.6	21.7	51.6	8.6	14.1	17.1	57.3	40.0	3.38
	6 (69S)	7.94	<1.25	12.9	25.4	82.4	9.4	14.9	26.8	83.8	16.9	4.28
	7 (70S)	8.22	<1.25	4.4	8.8	69.1	5.2	12.0	30.3	52.8	13.5	1.24
Normal limits		6.2 - 6.8	20.1 - 40.0	66.1 - 132.0	5.0 - 10.0	48.0 - 85.0	25.0 - 70.0	20.1 - 40.0	10.0 - 60.0	35.0 - 65.0	18.1 - 36.0	2.5 - 4

*physicochemical analysis results (HAGRO laboratory)

Samples from Arad County (Table 3)
In general, the soil is weakly alkaline (except for sample 12-94S which represents the reaction of a weakly acidic soil), well supplied with organic matter, but which in most cases has high-very high concentrations of sodium; it's about solonchak soils. If possible, amend the soil with gypsum - CaSO4.2H2O, phosphogypsum (residual material of phosphate fertilizer plants), calcium chloride - CaCl2.6H2O, Al sulphate, Fe sulphate, native sulfur or lignite powder.
- pH: soil reaction varies between weakly acidic (6.56) and weakly alkaline. Under these conditions, the more alkaline the pH, the lower the mobility and availability for plants of some nutrients: P, Mn, B, Zn and relatively Mg, Fe and Cu. The most affected is phosphorus, which will influence the growth of the root system, and together with boron the development of plants. Foliar fertilization with fertilizers rich in assimilable phosphorus and boron is recommended.
- ammoniacal nitrogen: it is relatively good; at the seedling stage, when the energetic state of the plants is precarious and in conditions of deficient solar radiation, however, higher values are recommended, but not more than 1/10 of the nitric nitrogen level.
- potassium: varies from very low (samples 8-90S, 10-92S and 12-94S) to very good (samples 9-91S and 14-95S; Figure 1). Where the values are very low-low, root fertilizers with potassium sulfate are needed, which will also correct the pH.
- calcium: normally should be at least equal to water-soluble potassium, if not even higher by 50%. Except for sample 10 (92S), the remainder reflects high concentrations of calcium. Analyzing these values compared to those of potassium, we can expect deficiencies in this last element.



Figure 1. Sample 12 (94 S – Arad) – normally developed tomato plants and Sample 14 (96 S – Arad) – tomato plants inhibited due to the high soil content of nitrites and nitrates

- magnesium: the optimal values, depending on the cultivated species, vary between 1/2 and 1/4 of the potassium values. Where the balance is in favor of magnesium, potassium deficiency may occur and vice versa. This is the case for samples 8 (90S) and 12 (94S). Due to its role in energy metabolism, protein, and especially nucleic acids, it increases the resistance of plants to pathogens (including fungi and bacteria). Its lack reduces the seed germination faculty.
- nitrites + nitrates: except for samples 10 (92S) and 12 (94S) which have low concentrations, the values are either normal or very high (sample 13-95S) or excessively high (sample 9-91S and especially 14 -96S).
- chlorides: varies from very low to excessively high (sample 10-92S and 9-91S and 14-96S respectively). Otherwise, the values are acceptable.
- sulfates: these are high values due to Ca, Mg and Na sulfates. There is, however, a beneficial effect. If there were lower values, we would probably have higher pH values.
- phosphorus: except for samples 8 (90S), 10 (92S) and 12 (94S), the other values are very high. Phosphorus deficiency increases the sensitivity of plants to: *Alternaria, Botrytis, Phytophthora, Fusarium* and *Erwinia* (case of samples 10-92S and 12-94S). High phosphorus levels facilitate the absorption of nitric nitrogen, which is high anyway. Excess of nitrogen may occur.

Table 3. The content in the main nutrients of soil samples from Arad county

Samples no. and code	Determinations performed*										
	pH	$N\text{-}NH_4$	K mg/kg	Na mg/kg	Ca mg/kg	Mg mg/kg	N mg/kg	Cl^- mg/kg	SO_4^{2} mg/kg	P mg/kg	Humus
8 (90S)	7.94	<2.5	48.4	367.6	580.0	103.8	247.5	68.8	3165.5	87.9	2.91
9 (91S)	7.56	<2.5	452.7	370.3	295.5	158.7	373.9	406.5	154.2	245.0	9.03
10 (92S)	8.31	<1.25	3.5	2.3	88.2	10.3	8.4	3.6	124.8	12.0	2.81
11 (93S)	7.64	<2.5	93.0	214.1	196.6	80.7	184.0	171.4	171.0	218.4	5.74
12 (94S)	6.56	<2.5	29.1	80.6	113.4	52.4	66.0	55.2	180.9	34.1	2.72
13 (95S)	7.62	<2.5	230.3	166.2	446.8	135.6	297.0	88.9	441.2	176.2	6.31
14 (96S)	7.46	<2.5	518.6	372.6	925.3	450.3	1007.3	440.1	275.5	239.9	6.31
Normal limits	6.2 - 6.8	20.1 - 40.0	66.1 - 132.0	5.0 - 10.0	48.0 - 85.0	25.0 - 70.0	20.1 - 40.0	10.0 - 60.0	35.0 - 65.0	18.1 - 36.0	2.5 - 4

*physicochemical analysis results (HAGRO laboratory)

Number of rising plants and frequency of attack of soilborne pathogens depending on the sample.
In the 14 soil samples (7 samples from Cluj county, 7 samples from Arad county) received for phytopathological analyzes, the presence of soil fungi *Pythium debaryanum, Phytophthora parasitica* and *Rhizoctonia solani* was detected, which cause "damping-off".

a. **Soil samples from Cluj County** (Table 4)
Depending on the soil sample, the number of cauliflower seedlings ranged from 62%

(sample 49S) to 100% (sample 50S; Table 4). The number of rotten seeds due to the attack of soil pathogens (*Pythium debaryanum, Phytophthora parasitica, Rhizoctonia solani*) varied between 0 (50S sample) and 38% (49S sample). In sample 49S the number of "fallen" plants was 2 (1 of *Pythium debaryanum* and 1 of *Rhizoctonia solani*) and in sample 50S the number of "fallen" plants was 3 (2 of *Pythium debaryanum* and 1 of *Rhizoctonia solani*). Depending on the soil sample, the number of plants grown on cabbage varied between 92% (sample 66S) and 100% (samples 67S and 70S). In sample 66S the number of rotten seeds, due to the attack of soil pathogens, was 8%, and in sample 66S 14%. In sample 66S the number of "fallen" plants was 13, of which 10 were due to the attack of *Pythium debaryanum* and 3 of *Rhizoctonia solani*. In sample 67S the number of "fallen" plants was 6, of which 5 were due to the attack of *Pythium debaryanum* and 1 of *Rhizoctonia solani*. In the 68S sample, the number of "fallen" plants was 6, of which 4 were due to the attack of *Pythium debaryanum* and 2 of *Rhizoctonia solani*. In the 70S sample, the number of "fallen" plants was 51, of which 44 due to the attack of *Pythium debaryanum* and 7 of *Rhizoctonia solani*. In the 69S soil sample, the number of sprouted celery plants was 70 and the number of rotten seeds was 30. In this sample, no “fallen” plants were registered. In the case of soil samples the 50S (seedlings 100, “fallen” plants 3) and 67S (seedlings 100, plants “fallen” 6) it can be estimated that they are slightly contaminated with soilborne pathogens.

Table 4. Number of plants raised and frequency [%] of attack of soilborne pathogens depending on the sample, Cluj County

Nr. crt.	Sample cod	Species tested	No. sprouted plants	Rotten seeds *)	"Damping-off" plants			
					Pythium debaryanum	*Phytophthora parasitica*	*Rhizoctonia solani*	Total
1	49S	Cauliflower	62	38	1	0	1	2
2	50S	Cauliflower	100	0	2	0	1	3
3	66S	Cabbage	92	8	10	0	3	13
4	67S	Cabbage	100	0	5	0	1	6
5	68S	Cabbage	86	14	4	0	2	6
6	69S	Celery	70	30	0	0	0	0
7	70S	Cabbage	100	0	44	0	7	51

*) Soilborne pathogen complex (*Pythium debaryanum, Phytophthora parasitica, Rhizoctonia solani*)

b. **Soil samples from Arad County**

Depending on the soil sample, the number of plants grown on peppers was between 84% (sample 92S) and 85% (sample 90S; Table 5). The number of rotten seeds due to the attack of soil pathogens varied between 15% (sample 90S) and 16% (sample 92S). In the 90S sample, the number of "fallen" plants was 5, of which 3 due to the attack of *Pythium debaryanum* and 2 of *Rhizoctonia solani*. In the 92S sample, the number of "fallen" plants was 2, 1 of which was due to the attack of *Pythium debaryanum* and *Rhizoctonia solani*. Depending on the soil sample, the number of plants grown on tomatoes was between 72% (sample 91S) and 95% (samples 94S and 96S). The number of rotten seeds, due to the attack of soil pathogens, was 28% (sample 91S), 21% (sample 93S), 5% (sample 94S) and respecitv 5% (sample 96S). In sample 91S the number of "fallen" plants was 60, of which 24 were due to the attack of *Pythium debaryanum* (Figure 2), 29 of *Phytophthora parasitica* (Figure 3) and 7 of *Rhizoctonia solani* (Figure 4).



Figure 2. *Pythium debaryanum* attack on tomatoes



Figure 3. Phytophthora parasitica attack on tomatoes

In sample 93S, the number of "fallen" plants was 19, of which 8 due to the attack of *Pythium debaryanum*, 9 of *Phytophthora parasitica* and 2 of *Rhizoctonia solani*.
No "fallen" plants were recorded in samples 94S and 96S.
In the 95S sample, the number of sprouted cucumber plants was 84% and the number of rotten seeds was 16%.

The number of "fallen" plants was 38, of which 32 were due to the attack of *Pythium debaryanum* and 6 of *Rhizoctonia solani*.
In the case of soil samples, 94S (rotten seeds 5, "fallen" plants 0) and 96S (rotten seeds 5, "fallen" plants 0) can be considered to be slightly contaminated with soilborne pathogens.



Figure 4. *Rhizoctonia solani* attack on tomatoes

Table 5 Number of plants raised and frequency [%] of attack of soilborne pathogens Depending on the sample, Arad County

Nr. crt.	Sample cod	Species tested	No. sprouted plants	Rotten seeds *)	"Damping-off"-plants			
					Pythium debaryanum	*Phytophthora parasitica*	*Rhizoctonia solani*	Total
8	90S	Bell pepper	85	15	3	0	2	5
9	91S	Tomatoes	72	28	24	29	7	60
10	92S	Hot pepper	84	16	1	0	1	2
11	93S	Tomatoes	79	21	8	9	2	19
12	94S	Tomatoes	95	5	0	0	0	0
13	95S	Cucumbers	84	16	32	0	6	38
14	96S	Tomatoes	95	5	0	0	0	0

*) Soilborne pathogen complex (*Pythium debaryanum, Phytophthora parasitica, Rhizoctonia solani*)

CONCLUSIONS

It is recommended to control of soilborne pathogen complex (*Pythium debaryanum, Phytophthora parasitica, Rhizoctonia solani*) with the following products:
1. Chemical products:
- Merpan 80 WDG (captan 800 g/kg) 0.15% (4-5 L solution/sqm) A maximum of 2 treatments are applied after sowing, at 14-day intervals (tomato seedlings).
- Previcur energy (propamocarb 530 g/L + fosetil 310 g/L) for tomatoes and cucumbers in protected spaces 3 L/ha applied by drip after transplanting (3-day break).
- Proplant 72,2 SL (propamocarb 722 g/L) 0.2% - 2 treatments are applied to the seedlings: the first at sowing - emergence 3-5 L solution/sqm and the second treatment one week before transplanting 3-5 L solution/sqm.
2. Biological products:
- Cavaler 600 SL (*Bacillus licheniformis* 1 x 10^7 ufc/ml + *Bacillus polymyxa* 1 x 10^6 ufc/ml + *Bacillus pumilus* 1 x 10^8 ufc/ml + Bacillus subtilis 1 x 10^8 ufc/ml) applied to vegetables by drip: solution of 1% (1L product in 100 L water) and foliar: 3L/hectar, 2 treatments, respectively 30 ml solution, in 10 liters of water/100 sqm.
- Garex B (garlic extract 80.73% + Bor 2%) 0.1-0.2% foliar application.

-Copfort (water-soluble copper 6% + copper complexed with gluconic acid 6%) 0.2% + Mimox Zn (Mimosa extract 80% + Zn 0.6% + Mn 1%) 0.3%.
-Algiforte (seaweed Ascophyllum nodosum + water-soluble magnesium oxide 1% + magnesium oxide complexed with gluconic acid 0.6%) 0.3% + Altosan (chitosan 4%) 0.3%
It is recommended to fertilize crops based on an integrated soil analysis service, from all points of view: physico-chemical (soil nutrient potential), phytopathological and microbiological (diseases and pests), from the point of view of pesticide residues remaining in the soil, pedological (sandy, loamy, clay soil, etc.).

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MORPHOLOGICAL AND AGRO-BIOLOGICAL CHARACTERIZATION OF LOCAL AND INTRODUCED GERMPLASM OF COMMON BEANS (*PHASEOLUS VULGARIS* L.).

Tsvetelina STOILOVA, Hristina RANGELOVA

Agricultural Academy, Institute of Plant Genetic Resources
2 Drughba Str., 4122, Sadovo, Bulgaria

Corresponding author email: tz_st@abv.bg

Abstract

Phaseolus germplasm collection has a considerable potential value which can be widely used in breeding work as well as for direct use by farmers. The objective of the present research was to make morphological, agro-biological and agronomic characteristics of local and foreign accessions in order to select those with desirable traits. The study was carried out during the period 2021-2023 with 35 accessions of common bean (Ph. vulgaris L.). The accessions were with maturity from 84 to 98, with mean value of 88.5 days. Six accessions were the earliest with 84 days, while the latest were with maturation of 98 days. Two accessions showed higher grain yield from one plant (C1E0076, 93201018) with 17.8 and 18.9 g, respectively. Bigger variation was registered in weight of plant without pods and weight of plant, as well as number of pods and weight of seeds per one plant with coefficient of variation (CV%) 94.8, 57.0, 51.2 and 44.5%, respectively. The promising accessions will be selected as the most perspective with a view of their future utilization in the breeding process.

Key words*: common bean, origin, germplasm, characterization, evaluation.*

INTRODUCTION

Among pulse crops dry beans (*Phaseolus vulgaris* L.) are one of the most important grain legumes. Common bean is cultivated in the tropics and the temperate zones and in many regions it is considered staple food (Adikshita & Kansak, 2017). *Phaseolus* sp. had been found a favorable climatic conditions and it is established a big *Phaseolus* diversity over the country with different morphological, agro-biological and agronomic traits (Genchev & Kiryakov, 2005). Common bean is a traditional crop for Bulgarian population and it is often grown in crop rotation with cereals because of nitrogen fixing fertilization and its consequence to the next crop (Zheng, 1995). Local varieties and populations are result of plant evolution with farmers' participatory selection and local agro-climatic conditions (Santalla et al., 1999; Miles, 2002). Challenges of climate change put bean plants in difficult conditions with extreme high temperatures and low rainfall during flowering and pod formation stages. The common bean varies morphologically in growth habit, plant height, number of pods per plants, weight of seeds per plant, weight of 100 seeds and seed characteristics. Phenotypic and genotypic characterization provides useful information for better utilization of germplasm collection. The farmers use these characteristics to distinguish their varieties and give them names (Mekbib, 2007; Loko et al., 2018). Genetic variation in bean's landraces is considerably high and has the most diverse population of cultivated crops (Frankel et al., 1995; Qualset et al., 1997). Landraces are a repository of valuable gene pool, local adaptation of domestic species, and further more are a great source of genetic variation which can be used for genetic improvement and future breeding activities (Nawaz et al., 2022). Because of the effect of climate change, old age of the farmers and depopulations of the territories there is a risk of extinction of local plant materials. *Ex situ* conservation in genebank protect the loss of agro-biodiversity and to maintain the genetic variation in response to environment conditions and crop management (Gomes et al., 2013). Bean landraces are shown wide genetic base with diverse reaction to abiotic and biotic stress factors, compared with modern bred cultivars with narrowed genetic base and more sensitive reaction to environmental challenges. In mung bean gene of bruchid-resistance has been

successfully transferred to the cultivated genotypes from wild mung beans (Zhu et al., 2012; Chen et al., 2015). The common bean collection preserved in the National genebank at the Institute of Plant Genetic Resources (IPGR) consist over 2200 accessions with local and foreign origin and this collection continue increasing number of accessions (Stoilova et al., 2014). At about 1/3 of the collection had local origin, while the rest of the accessions originated from different parts of the world. Scientific work on *Phaseolus* germplasm started in 1903 from the beginning of the Experimental station (Ann. Rep., 1904). Common bean germplasm differ according to the origin, specific production and life activities of the people (Long et al., 2020).
The objective of the present research was to make agro-biological and agronomic characterization of the accessions and their morphological traits with a view of their future utilization in the breeding process for bean improvement.

MATERIALS AND METHODS

The study took place from 2021 to 2023. Thirty five accessions with different geographical origin were characterized and evaluated in the experimental field of IPGR, Sadovo, Bulgaria (Table 1). Every accession was sown in two row plots and in two replications in a randomized complete block design. In each plot, 10 plants per replication were randomly chosen for biometric measurements. Observations were made for 16 morphological characters: 1) days to initial flowering, 2) days to mass flowering, 3) flowering duration, 4) days to maturity, 5) plant height, 6) weight of plant, 7) number of branches, (8) weight of plant without pods, 9) height of 1st pod, 10) number of pods/plant, 11) weight of pods per plant, 12) pod length, 13) pod width, 14) number of seeds per pod, 15) weight of seeds per plant, 16) weight of 100 seeds. Morphological observations were made on seeds, color and shape. The morphological characterization was done according to Phaseolus descriptors (Bioversity/IBPGR, 1983). Duncan's New Multiple Range Test (DNMRT) was used for mean separation. All data analyses were performed using Data collected in field trials were analysed by SPSS 19 (Core System User's Guide. 697).

Table 1. Bean accessions included in this study

No in row	Cat. No	Origin	No in row	Cat. No	Origin
1	88201014	Bulgaria (BG)	19	A9E0658	BG Devene vill.
2	88201009	Bulgaria	20	786091B	Bulgaria
3	B2E0282	China	21	97E0003	Bulgaria
4	B2E0283	China	22	A9E1054	BG (Radomir)
5	B2E0285	China	23	B0E0040	BG (Dimitrovgrad)
6	C1E0075	Egypt	24	A7E0156	BG (Trigrad)
7	C1E0076	Egypt	25	B9E0059	BG (Indge Voivoda)
8	94201001	Netherlands	26	B9E0009	BG (Petkovo vil.)
9	94201002	Netherlands	27	A7E0752	BG (Dubrovnitsa)
10	91210121	Spain	28	A8E0359	BG (Kableshkovo)
11	93201020	Hungary	29	78E6089	Bulgaria
12	91201182	Colombia (CIAT)	30	90 E0279	BG (Slavyanovo)
13	93201018	Hungary	31	90 E0305	BG (Lyatno vil.)
14	95201017	USA	32	90 E0128	BG (Lom)
15	B7E0208	Greece	33	B1E0480	BG (Velingrad)
16	A8E0514	BG (Ivailovgrad)	34	B1E0479	BG (Velingrad)
17	A8E0594	BG (Burgas)	35	C2E0034	BG (Krichim)
18	A9E1085	BG (Haskovo)			

RESULTS AND DISCUSSIONS

Six accessions, three with local and three with foreign origin (94201001, 93201020, 91201182, 786091B, B9E0009 and C2E0034) started initial flowering 47 days after emergency and entered in the mass flowering phase after 56 days (Figure 1).

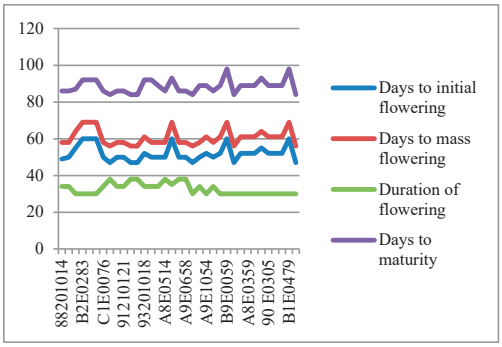


Figure 1. Phenological observations on 35 *Phaseolus* accessions

The earliest accessions matured after 84 days. Duration of flowering ranged from 30 to 38 days with mean value of 32.7 days. Accessions with foreign origin started mass flowering 58-61 days after emergency and matured for 84-92 days, while five accessions with local origin (B2E0283, B2E0285, C1E0075, A8E0594, B9E0059 and B1E0479) entered in full flowering stage after 69 days and reached

maturity in 92-98 days (Table 2). The difference of 47 and 69 days between the longest and shortest days to flowering and between 84 to 98 days to maturity showed a big variability in phenological phases among the all genotypes. This is in agreement with the results found by Fahad et al. (2014). Coefficient of variation (CV %) for the observed phenological stages were from 4.2% (days to maturity) to 9.4% (duration of flowering) (Table 2). The coefficient of variation (CV %) for days to initial and mass flowering were 8.2 and 7.2%.

Table 2. Minimum, maximum, mean and CV (%) values of phenological characteristics

Statistical values	Days to initial flowering (number)	Days to mass flowering (number)	Duration of flowering (number)	Days to 95% maturity (number)
Minimum	47	56	30	84
Maximum	60	69	38	98
Mean	52.1	60.7	32.7	88.5
St. Dev.	4.3	4.4	3.1	3.7
St. Error	0.7	0.7	0.5	0.6
CV%	8.2	7.2	9.4	4.2

Fifteen accessions were with indeterminate growth habit with plant height more than 75 cm. The maturation cycle of these accessions was 89-98 days with 5-10 days longer compared with accessions having determinate growth habit. The phenological differences exist because of genetically differences from each other which is in confirmation with reported results of 90 genotypes collected in Tanzania (Triphone & Nchimbi-Msolla, 2010).

Plant morphological traits (height of plant; weight of plant; number of branches; weight of plant without pods; height of 1st pod; number of pods /pl; weight of pods/plant) are shown in Table 3. Most of genotypes possessed determinate growth habit, which presented 57% of the total, 14.3% were with semi-climbing and 28.7% climbing growth habit. The tallest genotypes were predominantly with local origin with maximum value of the trait of 126.6 cm. The mean value of plant height was 69.5 cm with coefficient of variation (CV %) of 48.4%. Plants with taller stem (semi-climbing or climbing growth habit) showed potential for longer period of flowering and bigger seed yield. Tar'an et al. (2002) revealed positive correlations between components of plant height with time to flowering (FT) and rate of seed production. The most variable traits were, weight of plant and weight of plant without pods (with highest coefficient of variation CV-57% and 94.8%, respectively. Weight of plant differred among all accessions. The biggest value of weight of plant belonged to accession B9E0059 with 93.1 g the genotype with the least weight of plant was shown by accession C1E0075 with 12.2 g and mean value of the group was 30.6 g. In general phenotypic variation for agronomic traits ranged from 23.8 to 94.8% coefficient of variation (CV). Similar, high phenotypic variability of plant height and biological yield of 33 varieties and breeding lines was reported by Yohannes et al. (2020)

Table 3. Morphological characteristics of 35 bean accessions

Cat. No	h на p-e (cm)	Weight of plant (g)	Number of branches	Weight of plant without pods (g)	h 1st pod (cm)	Number of pods/pl.	Weight of pods/pl (g)
88201014	125,6	20,1	3,4	9,1	13,4	7,4	10,1
88201009	99,4	43,8	2,8	28,0	12,2	8,6	14,7
B2E0282	75,0	48,7	3,0	45,1	25,0	4,0	3,7
B2E0283	70,0	38,9	2,0	32,8	20,0	7,0	5,9
B2E0285	105,0	24,5	3,0	15,3	25,0	4,0	7,2
C1E0075	35,8	12,2	2,0	3,6	20,6	5,6	8,5
C1E0076	27,8	43,9	2,0	19,1	13,6	17,0	24,6
94201001	29,6	16,7	2,6	6,1	5,8	7,4	10,6
94201002	32,0	21,0	2,5	6,7	6,2	8,4	14,1
91210121	74,2	26,8	2,0	13,1	6,2	20,0	13,7
93201020	68,0	23,1	2,0	6,0	8,0	15,6	17,1
91201182	31,0	16,9	2,6	4,1	8,0	11,2	12,7
93201018	76,0	39,1	2,4	12,8	11,0	25,8	26,3
95201017	35,0	14,7	2,0	3,4	7,0	16,6	11,3
B7E0208	62,8	19,6	3,0	8,4	9,6	8,4	11,0
A8E0514	96,8	17,0	3,4	7,7	17,0	5,8	8,7
A8E0594	102,8	29,7	3,0	17,3	9,8	8,4	11,8
A9E1085	96,5	48,6	3,0	32,9	15,3	9,0	15,1
A9E0658	38,6	19,9	2,4	7,8	10,2	7,4	11,3
786091B	39,0	15,3	2,0	5,2	9,0	7,0	9,8
97E0003	35,6	20,5	2,0	12,2	9,8	6,0	7,7
A9E1054	38,2	30,0	2,2	15,1	11,6	10,4	14,0
B0E0040	62,4	15,8	3,4	8,4	14,4	5,8	6,5
A7E0156	39,6	21,0	2,8	14,4	15,4	7,0	6,8
B9E0059	121,0	93,1	4,0	82,2	11,3	6,7	9,9
B9E0009	122,4	75,1	4,0	66,1	13,4	5,4	8,4
A7E0752	29,6	29,9	3,0	12,7	11,6	13,2	15,8
A8E0359	126,6	32,8	4,0	25,4	12,4	5,4	7,0
78E6089	101,0	32,1	4,0	15,0	8,2	12,2	16,9
90 E0279	101,0	50,9	2,6	37,1	23,6	7,2	13,2
90 E0305	67,4	39,3	3,0	27,9	16,0	8,6	11,1
90 E0128	116,6	13,6	4,0	6,0	15,4	8,0	6,9
B1E0480	31,0	22,5	2,2	8,9	10,6	5,4	12,8
B1E0479	39,3	20,5	3,0	12,3	13,0	8,0	7,1
C2E0034	81,0	34,1	3,2	15,0	21,2	13,0	18,4
Min.	27,8	12,2	2,0	3,4	5,8	4,0	3,7
Max.	126,6	93,1	4,0	82,2	25,0	25,8	26,3
Mean	69,5	30,6	2,8	18,4	13,2	9,3	11,7
St. error	5,7	2,9	0,1	2,9	0,9	0,8	0,8
CV%	48,4	57,0	23,8	94,8	40,0	51,2	42,1

Seed yield is determined mainly by number of seeds per pod and seed weight per plant. In this study pod and seed size are presented in Table 4. The pod length varied between 6.4 and 13.7 cm, the biggest pod length belonged to accession A9E1085 and the least length of pod was mesured in B2E0282, both with local origin. The mean value of pod length was 9.6 cm with coefficient of variation (CV %) -17%. Pod width showed bigger variability compared with pod length with coefficient of variation (CV %) 22.5%.

Table 4. Pod and seed morphological traits

Cat. No	Pod length (cm)	Pod width (mm/cm)	Nr of seeds/pod	Seed length (mm)	Weight of seeds/pl (g)	Weight of 100 seeds (g)
88201014	7,9	1,1	4,6	0,9	7,5	30,4
88201009	10,3	0,9	3,8	1,2	9,4	31,4
B2E0282	6,4	1,8	2,0	1,2	1,2	26,3
B2E0283	6,7	0,7	3,0	0,9	3,3	26,3
B2E0285	9,0	1,4	3,0	1,2	5,0	28,9
C1E0075	9,0	1,0	3,2	1,3	6,5	33,0
C1E0076	10,7	0,9	5,8	1,0	17,8	39,5
94201001	8,7	1,2	3,2	1,2	7,5	38,0
94201002	8,6	1,4	3,2	1,5	9,7	61,0
91210121	9,1	0,8	4,5	1,2	9,4	22,7
93201020	9,5	1,2	3,6	1,4	11,7	37,2
91201182	11,2	0,8	3,8	1,5	8,6	45,0
93201018	8,7	1,2	3,4	1,4	18,9	33,5
95201017	6,8	0,9	4,0	0,9	9,1	17,9
B7E0208	9,8	1,0	4,6	1,2	8,5	29,3
A8E0514	10,1	1,0	3,8	1,1	6,3	33,9
A8E0594	9,3	0,7	4,4	1,1	8,0	33,1
A9E1085	13,7	1,1	4,8	1,4	10,5	23,2
A9E0658	11,5	1,2	7,2	1,1	7,5	33,2
786091B	9,8	0,9	5,0	1,1	6,6	21,4
97E0003	12,4	1,0	3,4	1,3	5,1	23,6
A9E1054	13,4	0,8	4,4	1,5	8,0	24,7
B0E0040	9,8	0,8	4,6	1,2	4,7	23,4
A7E0156	8,8	0,9	4,6	1,2	4,3	32,9
B9E0059	10,6	0,9	3,7	1,5	7,6	38,2
B9E0009	8,6	1,0	2,4	1,6	6,0	50,5
A7E0752	8,9	0,9	4,6	1,2	11,9	31,4
A8E0359	10,3	1,0	3,8	1,3	4,6	31,5
78E6089	9,7	1,0	6,4	1,0	11,2	24,5
90 E0279	8,0	0,7	4,4	1,0	10,3	21,1
90 E0305	9,0	0,8	4,6	1,2	8,2	26,9
90 E0128	10,2	0,9	4,6	1,1	5,1	24,4
B1E0480	10,4	0,9	4,4	1,1	8,8	31,6
B1E0479	9,0	1,0	5,3	1,1	4,8	26,8
C2E0034	8,6	0,9	4,6	1,2	13,4	34,0
Min.	6,4	0,7	2,0	0,9	1,2	17,9
Max.	13,7	1,8	7,2	1,6	18,9	61,0
Mean	9,6	1,0	4,2	1,2	8,2	31,2
St. Error	0,3	0,0	0,2	0,0	0,6	1,5
CV%	17,0	22,5	24,8	14,9	44,5	27,8

There were accessions with 0.7 mm (minimum) and with 1.8 cm (maximum). Number of seeds per pod varied between 2 to 7.2 with mean value of 4.2. Coefficient of variation was (CV%) 24.8%. Accessions with bigger number of seeds per pod were A9E0658 (7.2), 78E6089 (6.4), C1E0076 (5.8) with local origin. The seeds per pod in introduced common bean accessions were from 3.2 (94201001) to 4.6 (88201014). The positive correlation of grain yield with number of seed/pod and plant height was reported by Kamaludin and Ahmed (2011). The highest weight of 100 seeds were found by the genotype 94201002 with 61 g, followed by B9E009 (50.5 g) and 91201182 (45 g).

The plant height among all genotypes was divided in three statistical groups, low, moderate and hight plant height with level of sigificance at $P \leq 0.005$. First group contained 12 accessions of common bean with the shortest plants ranged between 29.6 and 39 cm (34.3%) (Table 5). The accessions with plant height situated in the medium group ranged between 39.6 and 77.8 cm (22.8%). The group with genotypes showed the highest plant height was formed by the four genotypes ranged between 119.6 and 126.6 cm (11.4%). There is a subgroup between medium and the highest plant height consisted by 11 genotypes with ranged of plant height from 81 to 116.6 cm (31.4%). Results showed five statistical groups among genotypes according to the height of the first pod (Table 5). The lowest pod was found in a group of eleven genotypes with pods located between 8.2 to 9.8 cm distance from the soil surface (31.4%). The highest pod was situated at 24.6 cm height from the soil. There were three subgroups, medium with ten accessions ranged from 10.6 to 13.4 cm, medium to high with two genotypes and the third subgroup with highest distance consisted of 7 genotypes (20%). According to the number of pods per plant genotypes showed three stitistical groups with significant differences. Accessions with the least number of pods per plant were 8 genotypes (22.8%), ranged from 4 to 5.4 (Table 5).

The medium group contained 7 genotypes (20%) with 6.4 to 7.4 number of pods per plant. Two genotypes showed the biggest number of pods per plant with 19.2 and 23.6, respectively. There is a subgroup between medium and highest number of pods per plant contained of 15 genotypes (42.8%). There is not so big diversity among all genotypes in weight of seeds per plant. There were two statistical groups with significant differences. In the first group were

two genotypes with 17.7 and 17.8 g, while in the second group were 33 genotypes (94.3%) with high weight of seeds per plant, ranged from 4.3 to 13.4 g. Weight of 100 seeds was divided in 4 statistical groups, the group with the least weight contained 14 accessions (40%), while the other three groups contained 2 or 3 accessions.

Table 5. Diversity among all included genotypes on five morphpological traits

Cat. No	Height of plant	Height of 1st pod	Nr. of pods/pl.	Weight of seeds/plant	Weight of 100 seeds
88201014	125,6a	13,4cd	7,4b	7,54b	7,5c
88201009	99,4ab	12,2cd	8,6ab	9,4b	9,4c
B2E0282	75,4b	24,6a	4,4c	1,61b	1,6d
B2E0283	72,4b	20,8ab	6,6b	3,15b	3,2d
B2E0285	101,2ab	23,2ab	4c	5,1b	5,1dc
C1E0075	35,8c	20,6ab	5,6c	6,5b	6,5c
C1E0076	27,8c	13,6c	17ab	17,8a	17,8ab
94201001	30,5c	7,2e	6,6b	7,2b	7,2c
94201002	33c	8,6e	7,6ab	9,3b	9,3c
91201121	73b	8,2e	19,2a	9,4b	9,4bc
93201020	69b	9e	14,6ab	11,4b	11,4ab
91201182	31,4c	7,6e	10,6ab	8,4b	8,4c
93201018	77,8b	11,6cd	23,6a	17,7a	17,7ab
95201017	38c	7,4e	17,4ab	9,6b	9,6b
B7E0208	108,8ab	9,6e	8,4ab	8,5b	8,5c
A8E0514	96,8ab	17ab	5,8c	6,3b	6,3c
A8E0594	102,8ab	9,8e	8,4ab	8b	8c
A9E1085	93,2ab	15bc	9,2ab	10,6b	10,6ab
A9E0658	38,6c	10,2d	7,4b	7,5b	7,5c
786091B	39c	9,4e	7b	6,7b	6,7c
97 E 0003	35,6c	9,8e	6bc	5,1b	5,1dc
A9E1054	38,2c	11,6cd	10,4ab	8b	8c
BOE0040	88,4ab	14,4bc	5,8c	4,7b	4,7dc
A7E0156	39,6b	15,4b	7b	4,3b	4,3dc
B9E0059	119,6a	11,2cd	6,4b	7,8b	7,8c
A9E1085	122,4a	13,4cd	5,4c	6b	6c
A7E0752	29,6c	11,6cd	13,2ab	11,9b	11,9ab
A8E0359	126,6a	12,4cd	5,4c	4,6b	4,6dc
78E6089	101ab	8,2e	12,2ab	11,2b	11,2ab
90 E 0279	101ab	23,6ab	7,2b	10,3b	10,3b
90 E 0305	67,4b	16ab	8,6ab	8,2b	31a
90 E 0128	116,6ab	15,4b	8ab	5,1b	31,4a
B1E0480	31c	10,6cd	5,4c	8,8b	26,8ab
B1E0479	39,8b	13cd	7,8ab	5,1b	26,3ab
C2E0034	81ab	21,2ab	13ab	13,4b	29a

There was a big subgroup between medium and highest weight of 100 seeds contained of 8 genotypes (22.8%) (Table 5).

Seed traits are the most important in common beans especially for commercial acceptability of different varieties (Bisht et al., 2014). The seed traits have important role as they are considered highly heritable (Blair et al., 2010). A big diversity among all studied accessions was observed, ranging from single color to mottled and striped types with different tonalities. White seed color was in 45.7% followed by mottled beige with lilac (14.3%) and brown color (11.4%). The rest groups were with less than 10% (Figure 2). There is a diversity in seed shape with predominantly kidney and cuboid shape, consisted 48.6% and 45.7%, respectively (Figure 3). The most frequent shape and color of bean seeds were noticed as white with kidney or cuboid shape.

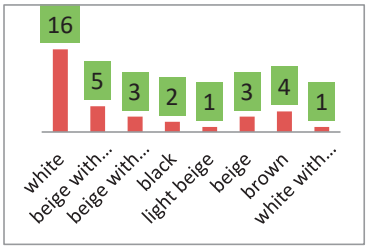


Figure 2. Seed color

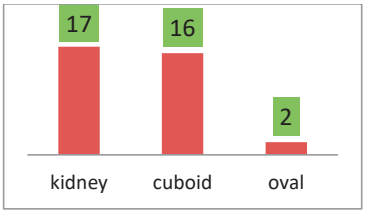


Figure 3. Seed shape

CONCLUSIONS

Morphological and agronomc traits were studied on a small sub set of Phaseolus vulgaris genotypes with local and foreign origin using qualitative traits. There is a diversity on the earliness of the genotypes, they can be divided as early, medium and late maturity. Accessions with cat. No 786091B and C2E0034 were the earliest with 84 days to maturity, while accessions with cat. No B1E0479 and B9E0059 were the latest with 98 days to reach maturity. Variability in morphological traits, plant height, heght of the 1 st pod, number of pods per plant, weight of seeds per plant and weight of 100 seeds were studied. Different statistical groups were established according to the variation of the trait inside the group of studied genotypes. The biggest diversity was found in the height of the first pod, followed by the weight of 100 seeds, plant height and weight of seeds per plant. The least variation was observed on the number of pods per plant. Accessions with cat. No B2E0282 and 90E0279 were with highest 1st

pod, accessions with cat. No 93201018 and 91201121 were with biggest number of pods per plant, while the biggest size of seeds were produced by the accessions with cat. No 90E0305 and C1E0034 with 31 and 29 g of 100 seeds. Diversity in seed color and shape was found with predominant white color and kidney shape.

The studied diversity among qualitative vegetative and reproductive traits can be used for breeding new varieties or for bean improvement.

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VEGETABLE MARKET EVOLUTION IN THE PERIOD 2009-2023 ORGANIC & CONVENTIONAL

Iulian Andrei SZEIKELY, Valerica TUDOR*, Laurenţiu Cătălin BOROIU, Răzvan Ionuţ TEODORESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: valericatudor@gmail.com

Abstract

Organic agriculture has experienced an accelerated development in recent decades in response to growing concerns about public health, food safety and environmental protection. Organic farming provides consumers with healthy food uncontaminated with toxic residues. The main objective of the present work was to investigate the evolution of organic & conventional vegetable farming at World, EU and Romanian levels over a period of fifteen years (2009-2023), in terms of cultivated areas, production, vegetable market analysis, situation of organizations and evolution of the certification process in vegetable crops (Romania), in a global context marked by the growth of this sector. Romania has a high potential in organic vegetable growing, but faces small areas, modest production and high dependence on imports.

Key words*: organic agriculture, trends, surfaces, producers, organic market.*

INTRODUCTION

Organic agriculture, unlike conventional agriculture, aims to preserve and even improve consumers' health along with the unaltered preservation of the environment by using friendly technologies, which preserve soil fertility, biodiversity and also minimize global environmental problems (Szeikely et al., 2024; Bonciu, 2022; Paull, 2023; Ioviță et al., 2024; Avasiloaiei et al., 2023).

In every region of the globe, organic agriculture action plans aim to develop the sector through increased production, demand and sustainability (Paull, 2023; Willer et al., 2021; Oshunsanya & Aliku, 2016; Fernandez-Cornejo et al., 1998).

Globally, but especially in Europe, a strong demand has emerged for obtaining agri-food products using clean technologies.

The organic sector has started to develop rapidly in the world, with consumers showing a real interest in reducing the risks that agricultural practices could cause to human health and the environment (Silva Dias, 2011).

Farmers in organic agriculture have as their main objective to produce, through environmentally friendly agricultural practices, high quality food with outstanding nutritional value and, above all, free from any health hazards for consumers (Fernandez-Cornejo et al., 1998).

The extent of organically managed agricultural land, the number of organic farms and the size of the global market for organically grown food have steadily increased (Willer et al 2017, 2021; Fawzy et al., 2016).

The expansion of organic production has involved producing nutritionally improved food crops while using fewer external inputs and reducing environmental impacts (Risku-Norja and Mäenpää, 2017).

In a 2022 study, organic farmland reached 72.3 million hectares (1.5% of all farmland), being managed organically by at least 2.7 million farmers in 187 countries. According to the study, the countries with the largest areas of organic land were: Australia (35.7 million hectares), Argentina (3.67 million hectares), Spain (2.35 million hectares), USA (2.33 million hectares) and India (2.30 million hectares), and the distribution of organic farmland by continent was: Oceania (50%), Europe (23%), Latin America (12%), Asia (8%), North America (5%) and Africa (3%) (Fernández et al., 2022; Paull J., 2023).

In the European Union, the Farm to Fork and Biodiversity strategies propose ambitious actions and commitments to combat biodiversity

decline, to transform food systems into global standards for competitive sustainability, the protection of human health and the environment, and to ensure livelihoods for all actors in the food value chain. A target has thus been set that by 2030, 25% of the EU's agricultural area should be used for organic farming (MADR, 2025). As technologies continue to permeate farming practices and the food chain, further exclusions can be anticipated to ensure that organic food is food, 'pure and simple', is what consumers would consider 'natural' and is food without the incursion of technopollutants and contaminants (Paull, 2023).

Organic is now an important aspect of consumers' food choices. The majority of consumers (72%) report buying organic foods "regularly" or "sometimes" according to a survey of 21,261 consumers in 38 countries (Nielson, 2005).

In this context, organic vegetables are an important component of the global agri-food system.

Organic farming systems differ fundamentally from conventional ones in their primary focus on management practices that promote and enhance ecological harmony (Tudor et al., 2024). In contrast to conventional marketing practices, almost half of the organic growers surveyed and the majority of the small organic growers market their vegetables directly to consumers through farmers' markets and other direct marketing channels (Brumă et al., 2023).

Certification of organic production provides certainty to consumers and recognition to farmers. It allows decoupling the relationship between producers and consumers as it preserves the organic identity and portability of these products differentiated in time and space. Certification paves the way for extending the supply chain, facilitates the supply of organic products to consumers at a geographical distance, not just within the country's borders, and also facilitates international exports.

Organic certification is a mechanism to monetize organic production by allowing product differentiation on the market. Certification maintains a price premium along the supply chain by preserving the organic identity of the product (PAN-UK, 2017).

With certification came logos and standards, the development of export opportunities and, over time, the mutual recognition of national certifications and standards, and eventually statistics and quantification of the organic phenomenon.

Although organic standards are specific to certification bodies, and certification bodies are usually local or national, there is a general harmony and congruence in the multitude of individual standards.

Certification favors large producers who distribute products widely. Organic certification favors large producers who distribute widely, while it disadvantages small producers with short supply chains, as certification costs can be discouraging or even prohibitive.

MATERIALS AND METHODS

The main objective of the present work was to investigate the evolution of organic vegetable farming at the World, European Union and Romanian levels over a fifteen-year period (2009-2023), in terms of cultivated areas, production, vegetable market analysis, situation of organizations and evolution of the certification process in vegetable crops, in a global context marked by the growth of this sector.

In this context, we resorted to a set of methods including documentation (analysis of scientific literature focused on this topic, at national, European and international level), data analysis of literature data, analysis and processing of data series from official national sources (Romanian Ministry of Agriculture and Rural Development, National Institute of Statistics - INS) and from international sources (FAO (FAOSTAT), EUROSTAT, FiBL (Research Institute of Organic Agriculture), IFOAM (International Federation for Organic Agriculture Movements), comparative analysis, synthesis and evolution of indicators over the mentioned time interval, and the results are illustrated in tabular form.

RESULTS AND DISCUSSIONS

The area planted to vegetables in the EU decreased significantly by more than 70% in 2023 compared to 2000, the main reasons including: fragmentation of vegetable land and small farm sizes, high and increasingly

expensive production costs (seeds, fertilizers, pesticides). The land cultivated with vegetables worldwide, both in conventional and organic systems, increased progressively over the period 2009-2023, with the areas cultivated in organic systems showing oscillating increases between 49757 thousand ha (2009) and 59132 thousand ha (2023) (Table 1). Within the EU, the organic farming system registers a significantly higher ratio of 7.7% (average 2009-2023), with 38.2% representing the EU/Global organic system ratio. In Romania, the areas cultivated with conventionally grown vegetables are decreasing, from 238 thousand ha (2009) to 90 thousand ha (2023). Although the areas cultivated in the organic system represent 1% of the conventional system, a slight increase can be observed in the last 3 years (2021, 2022, 2023), showing an increased interest in organic vegetable growing.

Analyzing the data in Table 1, a slightly increasing ratio of the area cultivated in the conventional system in Romania compared to the EU can be highlighted.

Table 1. Evolution of areas cultivated with vegetables, organic & conventional (thousand ha) (2009-2023)

Year	Worldwide		European Union		Romania	
	Conventional (thousand ha)	Organic (thousand ha)	Conventional (thousand ha)	Organic (thousand ha)	Conventional (thousand ha)	Organic (thousand ha)
2009	49757	219.3	2253	77.29	238	0.33
2010	51267	235	2162	81.52	236	0.73
2011	52355	250.52	2133	104.79	237	0.91
2012	53603	238.56	2037	77.99	232	0.89
2013	54549	293.18	2010	88.54	234	0.73
2014	55257	298.47	2035	97.4	219	1.91
2015	57029	318.99	2055	102.18	218	1.19
2016	56311	384.16	2105	129.51	208	1.16
2017	56800	379.84	2093	160.68	206	1.44
2018	56646	399.74	1671	181.18	117	0.97
2019	57163	430.72	1688	181.29	120	0.77
2020	57687	427.39	1697	195.4	98	0.83
2021	58263	475.97	1734	213.64	99	1.04
2022	58787	516.6	1590	213.32	88	1.04
2023	59132	551.93	1587	208.95	90	1.04
Average	**55,640.4**	**361.4**	**1,923.3**	**140.9**	**176.0**	**1.0**

Source: Own calculation based on the data from https://www.fao.org/faostat

Vegetable production generally requires a higher capital infusion than cereal crops and is often riskier as yields and prices are more variable. At world level, vegetable production increased by more than 70% in 2023 compared to 2009. At EU level, production is slightly fluctuating, with an average of 57,808.8 thousand tonnes (minimum 51672 thousand tons in 2023 and maximum 62,362 thousand tons in 2009). However, Romania shows a downward trend after 2015, dropping from over 3,200 thousand tons to below 1,200 thousand tons in 2023 (Table 2). This reduction can be correlated with the decrease in cultivated areas and with climatic, economic and technological factors.

Table 2. Evolution of vegetable production in the conventional system (million tons) (2009-2023)

Year	Worldwide	European Union	Romania
	(thousand tons)	(thousand tons)	(thousand tons)
2009	917654	62362	3259
2010	940817	59089	3214
2011	976623	60402	3546
2012	1002238	58366	2997
2013	1024323	57101	3344
2014	1059647	60092	3288
2015	1088202	59933	3184
2016	1091033	62355	2898
2017	1107210	62154	3102
2018	1108625	52636	2026
2019	1126758	54525	1853
2020	1144096	55867	1949
2021	1160501	58731	1939
2022	1171283	51847	1258
2023	1186682	51672	1199
Average	**1073713**	**57808.8**	**2603.7**

Source: Own calculation based on the data from https://www.fao.org/faostat

The global vegetable industry is now in a period of transition from increasing quantity to increasing quality and efficiency.
The vegetable grower needs to become more knowledgeable about vegetable crop production and adopt more convenient management techniques and cultural practices, such as irrigation and fertigation, protected cultivation and transplanting technology, and the use of better hybrid vegetable varieties.
Competition from imported vegetables from countries such as Turkey, Poland or the Netherlands, which are often cheaper than locally produced ones, is also a limiting factor.
Imports of vegetables (thousand tons) in the analyzed period (2009-2023), show an upward trend, with an average of 61956.5 thousand tons at world level, 21737.9 thousand tons in the EU and only 474 thousand tons in Romania, which represents an average of only 2.1 thousand tons compared to the EU (Table 3).
The export market (thousand tons) also has an upward trend, with a minimum ratio of 40 thousand tons between the EU and the quantities of vegetables exported worldwide (Table 3).
In Romania, the evolution of exports is upward starting from 41 thousand tons in 2009 and 104 thousand tons in 2022, with important fluctuations between years (Table 3).

Table 3. Evolution of imports and exports of vegetables in the conventional system (thousand tons) (2009-2023)

Year	Imports			Exports		
	Worldwide	European Union	Romania	Worldwide	European Union	Romania
	(thousand tons)	(thousand tons)	(thousand tons)	(thousand tons)	(thousand tons)	(thousand tons)
2009	48108	18392	254	50242	20747	41
2010	51442	18846	314	53382	21718	53
2011	53080	19490	313	55518	22957	57
2012	54023	19587	298	55383	23356	50
2013	56414	19905	307	58002	24091	61
2014	58817	20335	357	61649	24992	57
2015	60110	21076	414	61430	25096	57
2016	62508	21602	491	64308	25615	40
2017	63631	22431	512	66415	26926	51
2018	66603	23188	605	69163	27475	50
2019	68676	23736	611	70818	28016	49
2020	70282	23768	622	73649	28401	71
2021	70901	24296	652	74753	28360	78
2022	71975	24551	670	75342	28610	104
2023	72778	24865	693	74151	27206	99
Average	**61956.5**	**21737.9**	**474.2**	**64280.3**	**25571.1**	**61.2**

Source: Own calculation based on the data from https://www.fao.org/faostat

As global health awareness increases and household incomes rise, an increasing global demand for vegetables is expected.
As vegetable producers are typically better integrated into markets than field crop producers, vegetable crop production contributes to the commercialization of the entire rural economy, which is characterized by increased marketing and trade.
Studies show that the commercialization of vegetables stimulates and benefits the rural economy and contributes to the process of growth and development by generating employment and increasing agricultural productivity and secondary industries.
The value of exports and imports is directly proportional to production (thousand tons) (Table 4).
Table 4 shows that Romania imported vegetables worth 501.3 million USD (average 2009-2023), ranging from 217.1 million USD in 2009 to 993.4 million USD in 2023, with a difference of more than 25% between the value of imports and exports (Table 4).

Table 4. Evolution in value of imports and exports of vegetables in the conventional system (Million USD) (2009-2023)

Year	Imports			Exports		
	World	European Union	Romania	Worldwide	European Union	Romania
	(thousand USD)	(thousand USD)	(thousand USD)	(thousand USD)	(thousand USD)	(thousand USD)
2009	52922.3	24336.3	217.1	52748.2	27015.2	59.2
2010	59471.1	25689.9	239.5	59781.4	28952.5	86.6
2011	63753.5	26779.9	232.3	63851.1	29841.4	78.6
2012	62789.6	25999	236	62190.2	29856.4	75.8
2013	69883.1	28056.6	289.5	69087	32402.9	108.8
2014	71771.5	28641.8	361.8	73291.5	32741.3	130.1
2015	69779.1	26464	380	70199.4	29390.2	112.8
2016	73062.5	27942.7	472.2	74531.8	30783.7	95.4
2017	77535.2	30860.2	532.2	80097.7	33607.8	120
2018	80173.7	32165	634.3	83093	35551.6	140.5
2019	82434.9	32401.8	670.5	84242.1	35469	127.2
2020	87068.4	34577.2	692.5	87938.9	37830.1	151.7
2021	92948.2	37484.8	768.7	94292.8	41014.9	187.1
2022	98235.1	37861.9	800.2	97436.4	41052.7	221.8
2023	107018.9	44312.5	993.4	105847	46844.8	227.2
Average	**76589.8**	**30904.9**	**501.3**	**77241.9**	**34157.0**	**128.2**

Source: Own calculation based on the data from https://www.fao.org/faostat

We can conclude that globally and in European Uunion, imports and exports in the conventional system have increased both in volume (thousand tons) and value (million USD), reflecting an intensification of international trade in vegetables. In Romania, imports increased significantly, exceeding exports in volume and value, indicating a negative trade balance in this sector. Competitive participation in international markets requires relatively sophisticated marketing, information and transport networks as well as improved varieties, quality control, product standardization and, for some future markets, traceability. Other necessary improvements include pre- and post-harvest processing technologies, as well as market information systems including prices, seasonality information, post-harvest handling constraints and technological opportunities.

More than 2.7 million organic producers were reported worldwide, compared to 0.3 million in the European Union and only 0.01 million in Romania (average 2009-2023), which shows that Romania has a marginal share in the organic production sector at European and global level. This situation can be explained by a number of structural and conjunctural factors, such as the low level of information and training of farmers, insufficient specific financial support, the lack of well-established value chains, limited access to competitive outlets, and administrative and certification barriers (Table 5).

Table 5. Evolution of the number of organic producers, processors, importers and exporters (2009-2023)

Year	Organic producers (no.)			Organic processors (no.)			Organic importers (no.)			Organic exporters (no.)		
	Worldwide	EU	Romania	Worldwide	EU	Romania	Worldwide	EU	Romania	Worldwide	EU	Romania
2009	1806927	204417	3078	40707	31785	70	3023	2635	16	4157	10	0
2010	1564348	215472	2986	40070	31945	88	3147	2761	13	943	17	0
2011	1769447	231359	9471	44466	35201	106	9630	2913	2	3367	132	0
2012	1912045	249100	15315	49410	39416	105	3558	3020	3	2247	296	3
2013	1957379	252514	14901	53607	42862	100	3568	3015	1	2306	256	1
2014	2067063	252746	14159	62374	47890	124	3714	3065	3	4395	872	1
2015	2237937	265677	11869	68875	52178	142	4250	3510	6	6129	1957	2
2016	2544463	291721	10083	78428	57202	150	5279	3941	5	6338	2059	5
2017	2928406	302131	7908	86095	63384	161	6067	4504	9	7580	2676	6
2018	2784088	321772	7908	95216	69368	175	6571	5016	18	8678	3152	6
2019	3146851	338212	9277	100096	74866	191	7333	5559	24	8448	3130	15
2020	3494939	349509	9647	105072	77893	201	7783	5878	30	10007	3614	27
2021	3593062	384968	11562	113546	84452	209	8885	6642	34	11199	4126	25
2022	4514169	426688	12598	136813	87436	203	8483	6505	25	8426	4296	25
2023	4332499	434577	12598	133286	89379	203	10426	6727	25	9201	4898	25
Average	**2710241.5**	**301390.9**	**10224**	**80537.4**	**59017.1**	**148.5**	**6114.5**	**4379.4**	**14.3**	**6228.1**	**2099.4**	**9.4**

Source: Own calculation based on the data from: https://statistics.fibl.org

While data on organic producers are available for almost all countries, this is not the case for processors and importers, let alone exporters. Although data availability is improving, it is not yet possible to draw a clear picture to substantiate qualitative assessments and economic analysis, but only to present a punctual situation and a forecast of the situation for Romania. According to the data presented in Table 5, in 2023 the number of organic producers in Romania was 12598. In order to obtain and maintain the certification of organic production, each operator in organic farming undergoes a complete assessment of the activity, according to the inspection and certification contract concluded with the inspection body.

There are 15 control bodies (CBs) in the field of organic farming operating in Romania, approved by MADR on the basis of Articles 2-6 of MADR Order No 312/2021. On 11.07.2025, MADR published on its own website, the List of recognized producer groups with the following groups of products for which it obtained recognition: cereals, oilseeds, legumes, fodder, medicinal and aromatic plants, sugar beet, fruit and vegetables, potatoes, fruit and vegetables for processing, milk and dairy products, pig meat, beef, sheep, goat, poultry meat and eggs, propagation material (seeds, seedlings, cuttings, stolons, saplings, scions/grafts, rootstocks, etc.), wood, tobacco, honey and bee products, etc.

In terms of the structure of organic production and its share in total agricultural production, it can be observed that the highest share in organic production is held by cereals, oilseeds and fodder. The data show a geographical concentration of certifications in a few counties (Bihor, Arad, Constanța, Tulcea, Suceava), suggesting the existence of regional centers active in organic production (Table 6).

Table 6. Evolution of certifications for organic producer groups & organic vegetable producers in Romania, 2006-2025

COUNTY	Organic system		Organic vegetables	
	Recognition decisions (no)	Withdrawal decisions (no)	Recognition decisions (no)	Withdrawal decisions (no)
ALBA	7	6	0	0
ARAD	19	5	4	2
ARGEȘ	9	3	0	0
BACĂU	4	1	0	0
BIHOR	34	16	3	1
BISTRIȚA- NĂSĂUD	7	5	0	0
BOTOȘANI	5	2	0	0
BRAȘOV	7	3	4	1
BRĂILA	11	1	1	0
BUZĂU	18	4	1	1
CARAȘ SEVERIN	0	0	0	0
CĂLĂRAȘI	24	2	3	0
CLUJ	9	3	2	0
CONSTANȚA	16	4	8	2
COVASNA	22	6	1	0
DÂMBOVIȚA	10	6	6	5
DOLJ	5	3	3	3
GALAȚI	15	7	8	7
GIURGIU	8	0	4	0
GORJ	0	0	0	0
HARGHITA	8	1	1	0
HUNEDOARA	5	0	3	0
IALOMIȚA	13	5	3	2
IAȘI	6	2	1	0
MARAMUREȘ	7	5	4	3
MEHEDINȚI	2	1	0	0
MUREȘ	10	2	2	0
NEAMȚ	7	5	0	0
OLT	3	1	2	2
PRAHOVA	10	7	6	5
SATU MARE	15	4	1	1
SĂLAJ	7	2	0	0
SIBIU	5	1	0	0
SUCEAVA	20	10	5	0
TELEORMAN	7	4	1	1
TIMIȘ	13	2	2	0
TULCEA	21	3	5	0
VASLUI	2	2	0	0
VRANCEA	4	2	0	0
ILFOV	5	4	2	2
BUCHAREST	6	4	3	2
Total	**406**	**144**	**89**	**40**

Source: Own calculation based on the data from: https://www.madr.ro/grupurile-de-producatori-si- organizatiile-recunoscute-in-romania.html

From the data presented in Table 6, in the period 2006-2025, in Romania, 406 recognition decisions and 144 withdrawal decisions were granted, of which the vegetable sector accounted for 89 recognition decisions and 40 withdrawal decisions, which indicates a lower ratio, indicating difficulties in maintaining standards and economic sustainability of the groups and therefore the need for public support and technical assistance for farmers.

CONCLUSIONS

• Romania has a high potential in organic vegetable growing, but faces small areas, modest production and high dependence on imports.
• Organic certification is a certification of the production process, not a product certification.
• The organic value chain is incompletely developed, with few processors and exporters, which limits access to international markets.
• Public policies and financial support can play a decisive role in expanding and strengthening the organic sector, in particular by supporting local processing and facilitating access to markets.
• The exchange of good practices should be intensified.
• Simple and effective measures should be identified to increase the visibility of organic products (e.g., posters, website information)
• Improve product placement by using distinctive shelf labels (e.g., green labeling) and positioning organic products in prominent locations.

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PRESERVING BELL PEPPER QUALITY THROUGH FREEZING BY ASSESSMENT OF PRE-TREATMENT CONDITIONS AND FREEZE- DRYING EFFECT

Cristian-Alexandru TOADER[1], Mona Elena POPA[2]

[1]Vegetable Research and Development Station Buzău, 23 Mesteacănului Street, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Biotechnology, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: alexandru.toader@scdlbuzau.ro

Abstract

Bell peppers (Capsicum annuum) are a widely consumed vegetable; however, their highly perishable nature necessitates the implementation of preservation techniques to prolong their storage potential. This study sought to analyze and characterize the biochemical properties and freezing suitability of several bell pepper varieties, including Cantemir, Buzău 10, Arum, and Galben superior, which the Buzău Research and Development Station has approved for Vegetable Growing. This research endeavored to identify the bell pepper variety most suitable for long-term frozen preservation based on an evaluation of their physicochemical attributes. In order to determine the effect of the freezing process on the products, four sample variants were studied: fresh vegetables, immediately after harvesting; blanched vegetables; fresh vegetables, preserved by freezing and thawed at certain periods of time; blanched vegetables, preserved by freezing and thawed for certain periods of time. In this study, a series of analyzes and observations were conducted on the pepper fruits to assess various characteristics, such as humidity content, ascorbic acid content, total polyphenol content, acidity, and antioxidant activity at different maturity stages.

Key words*: bell pepper, varieties, freezing suitability, physicochemical properties.*

INTRODUCTION

To determine the effect of the freezing process on the products, four sample variants were studied: 1. Fresh vegetables, immediately after harvest; 2. Blanched vegetables; 3. Fresh vegetables, preserved by freezing and thawed at certain periods of time; 4. Blanched vegetables, preserved by freezing and thawed at certain periods of time. A series of analyses, biometric measurements and observations were carried out on pepper fruits, determining the following characteristics: humidity, total polyphenol content, acidity of the studied samples, determination of antioxidant activity at different degrees of maturity. In addition, observations were made in the crop regarding plant height, plant diameter, number of leaves/plant, leaf length, leaf width, number of buds, number of fruits, fruit length, fruit width, number of main branches, number of flowers and stem diameter. The results obtained revealed significant differences between varieties and treatments applied, with implications for the quality and durability of frozen peppers.

Bell pepper (*Capsicum annuum*) is an important vegetable, appreciated for its sweet taste and nutritional benefits. Freezing of vegetables is a widespread practice, but this process can influence the organoleptic characteristics and nutritional value of the final product. Thus, the aim of this study was to evaluate the effect of the freezing process on the quality of bell pepper varieties Cantemir, Buzău 10, Arum and Galben Superior, varieties approved by the Buzău Vegetable Research and Development Station (SCDL Buzău).

MATERIALS AND METHODS

The study was conducted during 2024-2025, within the Buzău Vegetable Research and Development Station and the Faculty of Biotechnology within the USAMV of Bucharest. To analyze the freezing suitability of peppers, four varieties of bell pepper (*Capsicum annuum* L.) were selected: Cantemir, Buzău 10, Arum and Galben Superior, all four varieties are approved by SCDL Buzău.

The varieties were cultivated in open fields, under agrotechnical conditions specific to the southeastern area of Romania. The cultivation technology included basic fertilization, drip irrigation, maintenance work and phytosanitary protection, according to current technological guides.

The fruits were harvested manually, when they reached commercial maturity, according to the standards specific to each variety. The samples were immediately transported to the laboratory, where they were sorted and prepared for the experiment.



VERDANT RED TWO-COLOURED

Figure 1. Appearance of Cantemir bell pepper variety



YELLOW TWO-COLOURED

Figure 2. Appearance of bell pepper variety Buzău 10



YELLOW

Figure 3. Appearance of the Yellow Superior bell pepper variety



RED

Figure 4. Appearance of Arum bell pepper variety

Freeze-drying preserved over 93% of the original polyphenol content in all varieties compared to the fresh sample. Also, antioxidant activity was significantly higher compared to frozen samples, indicating minimal degradation of phenolic compounds by this preservation method.

Freeze-drying, although a more expensive process than freezing, has shown significant advantages in preserving the nutritional and antioxidant value of bell peppers. Especially for varieties with high water content, such as Galben Superior, this method allowed for almost complete preservation of the initial biochemical profile. At the same time, the porous structure of the freeze-dried product favors rapid rehydration and maintenance of natural color, which makes it suitable for industrial applications or functional foods.

Experimental plan

To study the influence of freezing on the quality of bell peppers, four experimental variants were established for each variety:

1. **V1** – Fresh vegetables, analyzed immediately after harvest;
2. **V2** – Blanched vegetables (for 2 minutes in hot water at 95°C), then analyzed;
3. **V3** – Fresh vegetables, quickly frozen at -18°C, kept for 3 months, then slowly thawed under refrigeration conditions (+4°C);
4. **V4** – Blanched vegetables, frozen and stored at -18°C for 3 months, then thawed under the same conditions as in V3.

Freezing was performed by the quick-freezing method, using a forced-air freezer, to minimize the formation of large ice crystals that can affect the texture of the vegetable products. Thawing was performed slowly, in the refrigerator, to mimic home consumption conditions.

In addition to the freezing treatment variants, a fifth experimental variant was introduced for each variety studied:

V5 – Blanched vegetables, then freeze-dried

The freeze-drying process was carried out in a Labconco Freeze Dryer Freezone 2.5 laboratory freeze-drier

Labconco Freeze Dryer Freezone 2.5 at a pressure of 0.04 mbar and a shelf temperature of -50°C in the initial phase. The process took a total of 36 hours, until a constant mass was

reached. The fruits were cut into 1.5 cm cubes, after blanching (2 min, 95°C), to uniformize the drying.

Biochemical and physicochemical analyses

The following determinations were performed on samples taken from each variant:

- **Water content (%)**: determined by the oven drying method (105°C for 24 hours), calculating the difference between the initial mass and the mass of the dried sample.
- **Total polyphenol content (mg GAE/100 g)**: determined by the Folin- Ciocalteu method, using gallic acid as standard.
- **Total titratable acidity (g citric acid/100 g):** determined by titration with 0.1 N NaOH solution, to pH 8.1.
- **Antioxidant activity (% DPPH inhibition)**: analyzed by the DPPH method, expressed as percentage of free radical inhibition compared to a blank control.

Each analysis was performed in triplicate to ensure data accuracy.

Morphological and biometric determinations

In culture, observations and measurements were carried out on a sample of 10 plants for eachvariety, determining the following parameters:

- Plant height (cm)
- Plant diameter (cm)
- Number of leaves/plant
- Leaf length and width (cm)
- Number of buds and flowers/plant
- Number of fruits/plant
- Fruit length and width (cm)
- Stem diameter (mm)
- Number of main branches

The determinations were performed with standard laboratory instruments (millimeter ruler, electronic caliper, analytical balance, pH meter, UV-Vis spectrophotometer, etc.).

Statistical data processing

The results were statistically analyzed using Microsoft Excel, applying analysis of variance (ANOVA) to highlight significant differences etween varieties and treatments. Statistically significant differences were interpreted at a significance level of $p < 0.05$.

For each treatment variant, physicochemical and organoleptic analyses were performed. These included determination of moisture, total polyphenol content, acidity and antioxidant activity. In addition, biometric measurements of the plants and observations of the morphological characteristics of the fruits (length, width, number of fruits, stem diameter) were performed.

Parameters evaluated

For each variety studied, the following traits were analyzed:

- **Humidity**: determined by the gravimetric method to evaluate water losses during freezing and thawing.
- **Total polyphenol content**: measured by UV-Vis spectrophotometry, with the aim of evaluating the antioxidant power of peppers.
- **Acidity**: determined by titration, to evaluate pH changes during the freezing and thawing process.
- **Antioxidant activity**: evaluated using the DPPH (2,2-diphenyl-1- picrylhydrazyl) method, to understand the impact of processing on cellular protection.

In addition to these chemical analyses, biometric measurements and observations of the plants were also carried out, considering characteristics such as plant height, number of leaves, leaf size, number of buds and fruits, as well as aspects related to the main branches and flowers of the plant.

RESULTS AND DISCUSSIONS

The results showed that, regardless of the variety studied, freezing of vegetables led to a significant decrease in water content and changes in texture, especially in the case of samples that were repeatedly frozen and thawed. The Buzău 10 variety showed greater resistance to these changes, maintaining better consistency and nutritional value after freezing. In terms of antioxidant activity, blanched peppers retained a higher level of phenolic compounds than unblanched ones, suggesting that blanching is a beneficial process in preserving antioxidant properties.

Morphological analysis showed significant differences between varieties in terms of fruit size and vegetative development of plants. The Cantemir variety stood out with a higher fruit production, while Galben Superior had larger fruits but a lower number. Also, all varieties showed a significant increase in the main branches and the number of flowers in favorable growing conditions.

Interpretation of results:

-The average values obtained were compared between varieties and between treatments to highlight the influence of the freezing and blanching process on the physicochemical and nutritional characteristics of the fruits.

- Values marked with different letters in the resulting tables indicate statistically significant differences ($p < 0.05$) between treatments or varieties.

- The results obtained are consistent with the literature, supporting the hypothesis that heat treatment (blanching) followed by freezing has a beneficial role in maintaining antioxidant activity and fruit structure in some varieties.

- Freeze-drying produced visible changes in the physical structure of the fruits, but allowed for optimal preservation of bioactive compounds, especially in the Cantemir and Galben Superior varieties.

Table 1. Total polyphenol content (mg GAE/100 g dry product)

Variety	**V1 (Fresh)**	**V4 (Frozen + blanched)**	**V5 (Blanched + freeze- dried)**
Cantemir	95.2 ±2.1	82.4 ± 3.0	**90.5 ± 1.7**
Buzau 10	88.9 ±1.9	79.1 ± 2.8	**86.3 ± 2.1**
Arum	93.5 ±2.4	81.7 ± 2.5	**89.0 ± 1.5**
Yellow Superior	96.8 ±1.8	83.6 ± 3.1	**94.7 ± 1.9**

Table 2. Antioxidant activity (% DPPH inhibition)

Variety	**V1 (Fresh)**	**V4 (Frozen + blanched)**	**V5 (Lyophilized)**
Cantemir	72.1 ±2.2	60.8 ± 2.5	**70.2 ± 1.6**
Yellow Superior	74.5 ±1.9	63.3 ± 3.0	**73.1 ± 1.7**

CONCLUSIONS

Freezing vegetables is an effective method of preservation, but its effects on quality and nutritional properties vary depending on the variety and treatments applied. Following the research, the varieties Buzău 10 and Arum demonstrated the best results in terms of preserving quality and nutrient content after freezing. The study suggests that blanching before freezing and observing optimal thawing periods can significantly contribute to preserving the biochemical properties of bell peppers.

Freeze-drying has been shown to be a superior preservation method compared to freezing, preserving a higher proportion of phenolic compounds and antioxidant activity. Thus, for premium products or products destined for the specialized food industry, freeze-drying could represent a valuable alternative to freezing, especially for varieties sensitive to thawing.

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PRODUCTIVITY PARAMETERS OF SWEET CORN CULTIVATED UNDER METEOROLOGICAL CONDITIONS OF PLOVDIV

Violeta VALCHEVA[1], Kostadin KOSTADINOV[1], Nadezhda SHOPOVA[2], Radoslav CHIPILSKI[3]

[1]Agricultural University - Plovdiv, AU, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria
[2]Climate, Atmosphere and Water Research Institute at Bulgarian Academy of Sciences (CAWRI-BAS), 66 Blvd Tzarigradsko chausse, 1784, Sofia, Bulgaria
[3]Institute of Plant Genetic Resources, 2 Drouzhba Str., 4122 Sadovo, Plovdiv District, Bulgaria, Agricultural Academy, Sofia, Bulgaria

Corresponding author email: kostadinov8888@gmail.com

Abstract

Sweet corn falls into the group of vegetable crops with great sensitivity to environmental conditions. In climate change conditions, the manifestations of its productive parameters arouse high scientific interest due to the wide application of this crop. Therefore, the purpose of this study is a comparative analysis of four promising sweet corn varieties for Bulgaria - Zeaton F1, Turbo F1, HMX5389 F1 and HMX59YS832 F1. The experiments were carried out under the meteorological conditions of 2023 and 2024 on the Agricultural University of Plovdiv territory. The results obtained provide information about the productive and morphological parameters of the varieties. The variety with the shortest vegetation period, Turbo F1, has the best productive parameters: ear mass, number of rows in the ear, number of grains per ear, grain weight per ear, weight of 1000 grains and length of the grain. The correlation analysis calculated the strongest relationship between ear mass and both the kernel weight per ear and the mass of 1000 kernels.

Key words*: productivity parameters, sweet corn, heat stress.*

INTRODUCTION

Sweet corn (*Zea mays* var. *saccharata*) is a staple crop of significant economic and nutritional importance. It is a vital crop globally, valued for its sweetness, tender kernels, and nutritional benefits. The growing demand for sweet corn in fresh, canned, and frozen forms underscores the importance of improving productivity parameters to meet consumer needs. Its unique flavour, high sugar content, and versatility in both fresh and processed markets highlight the necessity of enhancing productivity. The productivity of sweet corn cultivation is influenced by multiple factors, including genetic traits, agronomic practices, and environmental conditions, with meteorological factors playing a crucial role in determining crop performance and yield outcomes (Morton et al., 2017; Popova et al., 2021). Productivity parameters are significantly affected by climatic and meteorological conditions, which shape growth, development, and yield potential. Plovdiv, Bulgaria, is characterized by its diverse weather patterns, making it an ideal case for studying the effects of these variables on sweet corn cultivation. Climate variability and change have an increasing impact on agricultural productivity worldwide. The influence of meteorological factors on crop productivity has been extensively studied.

The effects of temperature and rainfall variability on maize yields globally are examined (Smith et al., 2020). Changes in climate patterns influence phenological development and productivity, providing a comparative basis for understanding regional impacts.

Focusing on the Balkan region, it is identified that key climatic factors influence sweet corn production (Petkova et al., 2018). The changes and agrarian reform in Bulgaria since 1989 bring many risks and challenges to agriculture and irrigation opportunities (Kolcheva, 2024).

The interaction between irrigation practices and climatic conditions has been explored, demonstrating the significance of water

management in mitigating the effects of climate variability on sweet corn yields (Chen et al., 2019).

Agrometeorological models tailored to Bulgarian conditions have been developed, offering insights into optimizing crop productivity under specific environmental scenarios (Georgieva et al., 2021). The phenological responses of sweet corn to climatic stressors such as heat waves and drought have been investigated, highlighting the need for adaptive strategies to sustain yields (Rahman et al., 2017).

Research into future climate scenarios shows how maize yields in the Midwest may be affected, emphasizing the role of climate variability in yield fluctuations and offering insights applicable to sweet corn (Southworth et al., 2000).

The effects of climate variability on maize yields, particularly drought episodes and their impact on productivity, have been analyzed, providing a comparative framework for assessing sweet corn's sensitivity to similar conditions (Omoyo et al., 2015).

Temperature and rainfall have been shown to significantly influence seasonal maize yields, offering valuable insights into seasonal climate impacts on sweet corn (Wang et al., 2022). Climatic stress factors such as rainfall variability and temperature extremes have been identified as key determinants of maize yields, shedding light on how sweet corn might respond to similar meteorological conditions (Cudjoe et al., 2021). A methodological framework for assessing these effects on sweet corn in regions like Plovdiv has also been proposed (Mumo et al., 2018). The influence of temperature and rainfall variability on yields has been emphasised, underlining their importance for sweet corn cultivation (Attia et al., 2022).

Projections of future maize yield scenarios under climate change offer a long-term perspective relevant to developing adaptive strategies for sweet corn (Jones et al., 2003). Using a generalized least squares model, it is examined how climate variables influence maize yields over time, providing insights for modelling sweet corn productivity (Wu et al., 2021).

Maize yield vulnerability under various climate scenarios has been explored, contributing to a better understanding of adaptive measures applicable to sweet corn (Shi & Tao, 2014). The interaction of rainfall and temperature changes on maize yields, the need for adaptive measures in agricultural practices (Oseni & Masarirambi, 2011).

Changes in rainfall patterns are serious challenges (Liu et al., 2023). Heat and drought have been reported to increase osmotic stress and seed germination, plant growth, leaf expansion and ear development (Revilla et al., 2021). When heat stress is present during ear differentiation, there is a reduction in ear length and kernel row number. When heat stress is present during tasseling, there is a significant reduction in ear weight (Nemeskéri et al., 2019). Climate change and weather variability have affected the growth and development of vegetable crops worldwide (Abewoy, 2018). The impact of weather variability is further enhanced by the frequent use of supersweet corn varieties, which have the highest potential yield but are most sensitive to drastic changes in daily air temperature and soil water availability (Nemeskéri et al., 2019).

There are studies about how environmental factors, particularly temperature and relative humidity, influence key productivity parameters such as cob size, kernel weight, and overall yield in sweet corn (Tas and Mutlu, 2021).

The importance of understanding these relationships is emphasised as key to optimising cultivation practices and ensuring sustainable production under varying climatic conditions.

The need for region-specific studies is also highlighted to develop tailored cultivation practices that maximise yield potential across different climate scenarios. This study aims to evaluate the productive parameters of several sweet corn hybrids grown in Plovdiv under temperature stress conditions at a later sowing date. Understanding of critical phases and appropriate hydrothermal windows during the hot summer period is discussed. The study will help farmers to select suitable hybrids, optimal sowing dates, as well as good agronomic practices. Understanding plant responses to environmental conditions and selecting the most adaptable variety is the first step in developing the best management practices for sweet corn production in the region.

MATERIALS AND METHODS

The experimental work was undertaken in the period 2023-2024. In the field of AU-Plovdiv, with four sweet corn hybrid varieties: Zeaton F1, Turbo F1, HMX5389 F1 and HMX59YS832 F1, with a sowing date of June 1. The plants were sown according to the scheme 70/20 cm. The experiment was carried out according to the block method in 4 variants with four repetitions, with 50 plants per repetition, and the size of the experimental plot was 7 m^2. Watering was carried out with a drip system. The following variants were tested: 1. Zeaton F1 - Control; 2. Turbo F1, HMX5389 F1; 3. HMX5389 F1; 4. HMX59YS832 F1. The plants were fertilised with Gold Forte – organo-mineral fertiliser containing N 48%, P 8%, K 4%, Mg 2%, Mn 0.4%, Mo 0.02%, Zn 2%, Free acids 6%, Alginic acid 0.2%, Gibberellic acid 150, pH: 4.5-6.5. Fertilisation was applied at a rate of 1000 ml/da, four times in vegetation- 2-leaf stage, 6-leaf stage, before tasselling, and during grain filling. The productivity of the plants was determined on 12 plants of a variant in consumer maturity in September by taking into account the total yield. The morphological characterization of the ears was done by determining the parameters ear length (cm), ear diameter (cm), ear mass (g), number of rows in the ear, number of grains in the ear, weight of grains in the ear (g), weight of 1000 grains (g), length of grain (cm), width of grain (cm).

Meteorological data. For this experiment, observations were organised on the hourly values of basic meteorological parameters. The data were collected using an automatic station located next to the experimental field. Data were processed for: maximum air temperature (°C), relative air humidity (%), wind speed in m/s meters per second at 2 meters above the ground surface, and humidity of the root-dwelling soil layer. The base temperature for corn development was assumed to be 10° C. The plants were grown under drip irrigation.

Statistical analysis of the data was performed using one-way analysis of variance (ANOVA), Fisher's least significant difference (LSD) test and correlation analyses.

RESULTS AND DISCUSSIONS

Sweet corn is very sensitive to high temperatures. Its entire cycle from sowing to harvest is significantly influenced by weather conditions. Some studies (Dhaliwal et al., 2022) show that temperatures during the growing season above 30 °C were detrimental to crop yields. Each additional degree day spent above 30°C during flowering reduces yields by 0.5% and 2% in irrigated and rainfed fields, respectively. Sweet corn is most vulnerable to heat waves during its reproductive period. In the first year, the most stressful conditions are observed in the last ten days of July, when air temperatures above 40°C, relative humidity below 25% and wind speeds at 2 meters above the ground surface of about 5 m/s are recorded. Air temperatures above 35°C prevail throughout the month (Figure 1).

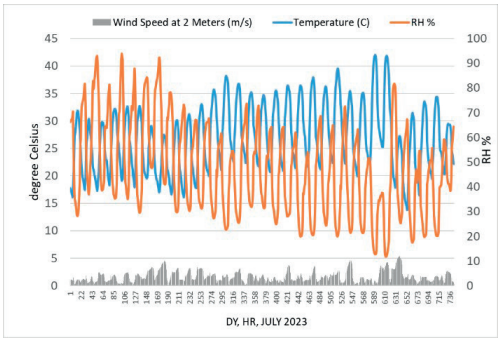


Figure 1. Distribution of Maximum Air Temperature (°C), Relative Humidity (%), and Wind Speed (m/s) by Hour in July 2023

Slightly lower values, around 30°C-32°C but with humidity between 85% and 95%, were recorded at the beginning of the month. In 2023, the least unfavourable period for the reproductive period of sweet corn was the last days of the first and last ten days of the month. Conditions in July 2024 remain highly stressful, with better periods for the critical phases of sweet corn cultivation observed at the beginning and end of the month (Figure 2). A significant number of hours with air temperatures above 32°C and 35°C were recorded, mainly in the middle of the period.

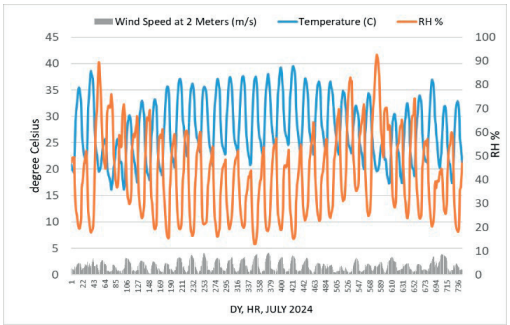


Figure 2. Distribution of Maximum Air Temperature (°C), Relative Humidity (%), and Wind Speed (m/s) by Hour in July 2024

In August, the hydrothermal conditions in the experimental area are slightly more favourable compared to those of the previous July (Figure 3). The middle of the period is most suitable for pollination. Throughout August 2024, there are a significant number of hours with relative air humidity below 25% (Figure 4).

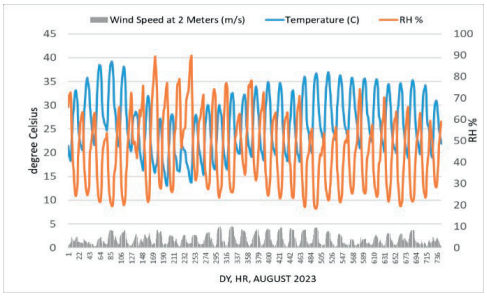


Figure 3. Distribution of Maximum Air Temperature (°C), Relative Humidity (%), and Wind Speed (m/s) by Hour in August 2023

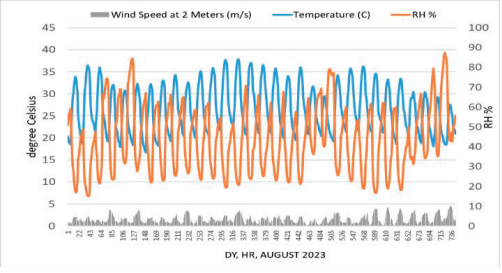


Figure 4. Distribution of Maximum Air Temperature (°C), Relative Humidity (%), and Wind Speed (m/s) by Hour in August 2024

During the critical phases at the end of the second decade, temperatures are slightly lower, and the wind is weakest.

During the two experimental years, a significant number of days with maximum temperatures > 32°C were observed, with more cases in 2024 than in 2023 (Table 1).

The cumulative accumulation of effective temperatures is shown in Figure 5.

Table 1. Number of days with maximum temperatures > 32°C in different intervals during the period June-August

Period/Temp.° C	32-34	34-36	36-38	38-40	>40
(VI-VIII) 2023	19	17	11	1	1
(VI-VIII) 2024	30	28	19	3	1

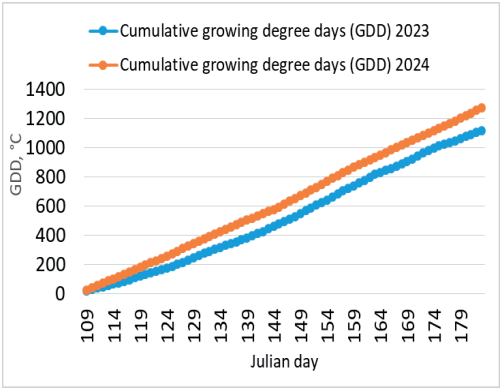


Figure 5 Cumulative growing degree days during experimental period

Growing degree days (GDD) are calculated by subtracting a base or threshold temperature from the average daily temperature. For corn, this base temperature is 10 degrees Celsius. The cumulative GDD10 shows year-to-year variability. The critical phenophases occurred around mid-July, depending on the hybrid.

The vulnerability of maize to drought in the Plovdiv region is very high due to the lower total available water (TAW = 116 mm) (Popova, 2015). The same authors determined net irrigation requirements (NIR, mm) for maize relative between 280 mm and 400 mm depending on the moisture conditions. Unlike grain corn, sweet corn is a vegetable crop, and irrigation is mandatory for yield. Soil moisture in the experimental plot was maintained in an optimal mode during both experimental years through the capabilities of a drip irrigation system (Figure 6).

The additional water provided significantly mitigated the adverse effects of heat waves on the studied plants.

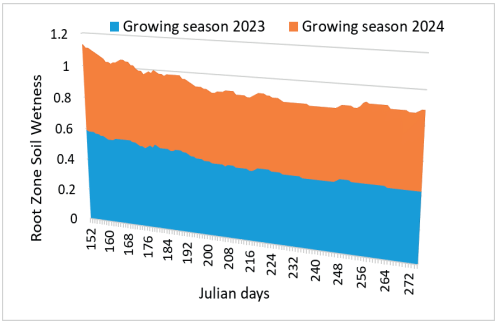


Figure 6. Root zone soil moisture wetness from June

Table 2 presents the two-year average values of biometric parameters of ear, grain and productivity recorded after maturation. The length and diameter of the ears vary slightly. The variety HMX5389 F1 has the longest ear, while the control variety Zeaton F1 has the shortest, with no statistically significant difference between them. For the other parameter, a significant difference of up to 5% is observed only between HMX5389 F1 and Turbo F1, but compared to control Zeaton F1, there is no significant difference.

For all other productivity parameters, the differences among the varieties are more pronounced and statistically significant. Regarding ear mass with grain, Turbo F1 has the highest mass and, along with HMX5389 F1, belongs to the same group. The control variety Zeaton F1 has the lowest ear mass, with a statistically significant difference from the above-mentioned varieties. The results for the parameter number of rows per ear, number of grains per ear, and grain weight per ear give advantage to Turbo F1, which exceeds the next varieties, HMX5389 F1 and HMX59YS832 F1, by an average of 15%. The greatest difference between Turbo F1 and the least productive variety Zeaton F1 is observed in the number of grains per ear, where all other varieties have a statistically significant difference of up to 5% compared to the control variety Zeaton F1.

For the indicator mass of 1000 grains, the trend in values is maintained, but with the difference that in HMX59YS832 F1, the higher number of seeds per ear leads to a lower mass of 1000 grains, which is the opposite of HMX5389 F1. In the most productive variety, Turbo F1 and the least productive control variety Zeaton F1, this inverse relationship is not observed.

The morphometric parameters of the grain indicate an advantage in grain length for the higher-yielding varieties Turbo F1, HMX5389 F1, and HMX59YS832 F1. Regarding grain width, HMX59YS832 F1 has the highest value, followed by Zeaton F1. Interestingly, there is a statistically significant difference between HMX59YS832 F1 and the high-yielding Turbo F1, which has the smallest diameter.

Table 3 presents the correlation dependencies between productivity parameters recorded after maturation. Parameters characterizing the ear, namely length, mass with kernels, and the number of rows, show a moderate to strong positive correlation with productivity indicators. The strongest relationship is calculated between ear mass and the kernel weight per ear and the mass of 1000 kernels. A similar strong correlation is reported by Agapie and Sala (2023).

A moderate correlation is observed between ear length and the indicators kernel weight per ear and mass of 1000 kernels, as well as between ear mass and the number of kernels per ear and the number of rows with kernel weight per ear. Agapie and Sala (2023) report correlations of the same strength. All of the above interactions are statistically confirmed at the 5% and 1% significance levels.

For the morphometric indicator kernel diameter, no significant positive correlation is calculated with the elements of productivity.

The strongest positive correlations among the morphometric indicators of the ear are calculated for ear length with ear mass and ear mass with the number of rows per ear. Among the productivity indicators, the most notable relationships are between kernel weight per ear and the mass of 1000 kernels, as well as the number of kernels with kernel weight per ear.

From the calculated correlation dependencies for the morphometric parameters of the kernels, only kernel length shows a strong positive and statistically significant impact on productivity and ear morphometry. The other indicator, kernel width, similar to ear diameter, does not influence any of the productivity indicators examined in the study.

Table 2. The productivity parameters of corn hybrids varieties

Parameters	Ear length (cm)	Ear width (cm)	Ear mass (g)	Number of rows in the ear	Number of grains in the ear	Weight of grains in the ear (g)	Weight of 1000 grains (g)	Length of grain (mm)	Width of grain (mm)
Zeaton F1-control	19.83	4.37	112.33	16.00	595.67	91.33	142.67	9.93	5.00
Turbo F1	20.67 n.s.	4.67 n.s	175.33 **	17.67 n.s	737.33 **	158.00 **	211.00 *	12.38**	4.07 n.s.
HMX5389 F1	21.33 n.s	3.97 n.s	153.00 *	16.00 n.s	698.67 *	128.67 n.s.	185.33 n.s.	11.00n.s.	4.87 n.s.
HMX59YS832 F1	21.00 n.s	4.37 n.s	143.00 n.s	16.00 n.s	722.67 **	111.00 n.s.	156.00 n.s.	11.67 *	6.17 n.s.
GD 5% *	2.23	0.51	36.93	1.69	98.46	39.96	59.35	16.4	1.46
GD 1% **	3.09	0.70	51.25	2.35	136.66	55.46	82.38	22.8	2.03
GD 0.1% ***	4.30	0.98	71.28	3.27	190.08	77.14	114.58	31.7	2.82

n.s. no significant difference

Table 3. Correlation coefficients between productivity parameters of corn hybrids varieties

Parameters	Ear length (cm)	Ear width (cm)	Ear mass (g)	Number of rows in the ear	Length of grain (mm)	Width of grain (mm)	Number of grains in the ear	Weight of grains in the ear (g)	Weight of 1000 grains (g)
Ear length (cm)	1.00	0.21	0.59*	0.05	0.52*	0.07	0.28	0.52*	0.59*
Ear width (cm)		1.00	0.32	0.29	0.51	-0.46	0.24	0.33	0.28
Ear mass (g)			1.00	0.54*	0.89**	-0.29	0.56*	0.98**	0.93**
Number of rows in the ear				1.00	0.60*	-0.49	0.51	0.63*	0.46
Length of grain (mm)					1.00	-0.35	0.52*	0.88**	0.82**
Width of grain (mm)						1.00	-0.20	-0.39	-0.28
Number of grains in the ear							1.00	0.53*	0.28
Weight of grains in the ear (g)								1.00	0.94**
Weight of 1000 grains (g)									1.00

*-correlation is significant at the 5%; **- correlation is significant at the 1%

CONCLUSIONS

The productive indicators of sweet corn in the Plovdiv region, grown at a late sowing date under conditions of high thermal stress, were studied. The experiment was conducted in two of the warmest years since 2020. During the growing season, 41 days with maximum temperatures > 32°C were recorded in 2023 and 81 days in 2024. Although 2024 is hotter and drier, pollination conditions are in a more favourable window compared to those in 2023. Under irrigation conditions, all observed hybrids have given good results. The variety with the shortest vegetation period, Turbo F1 has the best productive parameters: ear mass, number of rows in the ear, number of grains per ear, grain weight per ear, weight of 1000 grains and length of the grain. Irrigation has significantly mitigated the unfavourable hydrothermal conditions in the reproductive phases of sweet corn development. Therefore, temperature stress and increased evaporation lead to the need for higher irrigation rates.

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IMPROVING PLANT CANOPY MICROENVIRONMENT CONDITIONS BY USING DOUBLE CHANNEL AERATION CULTIVATION SYSTEM IN A PLANT FACTORY: SIMULATION ANALYSIS AND EXPERIMENTAL STUDY

Yihan ZHANG[1], Florin STĂNICĂ[2], Ruifeng CHENG[1]，Yuxin TONG[1]

[1]Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: tongyuxin@caas.cn

Abstract

To address the current issues in plant factories including stagnant airflow in plant canopies, uneven distribution of environmental factors, and high complexity of environmental control systems, this study developed an integrated double-channel aeration cultivation system (DCAS) combining ventilation ducts with cultivation tanks. A three-dimensional computer fluid dynamic model was developed and validated through simulating the airflow distribution within the plant canopy under different ventilation parameters. According to the simulated results, a ventilation angle of 45° with an intake air velocity of 8 m s^{-1} showed the best airflow uniformity and highest proportion of suitable zone. Based on the optimized parameters, a lettuce cultivation experiment was conducted in a plant factory. The experiments investigated the effects of traditional ventilation mode and DCAS ventilation on lettuce growth, canopy environmental factors and heat exchange parameters. The experimental groups used DCAS ventilation with canopy airflow velocities set at 0.6 m s^{-1} (T_1), 0.9 m s^{-1} (T_2) and 1.2 m s^{-1} (T_3) respectively, while the control group (CK) used the conventional side-inlet top-outlet ventilation mode with canopy airflow velocity of 0.2 m s^{-1}. Results showed that compared with CK, the experimental groups demonstrated better performance in lettuce growth, canopy microenvironment and heat exchange with the surrounding environment. Under T_2 treatment, the dry and fresh weights of lettuce shoots increased by 24% and 14%, respectively, compared to those under the CK. The canopy microenvironment and heat exchange reached optimal status under T_3 treatment, where the average relative humidity and air temperature in lettuce canopy decreased by 8.8% and 2.8°C, respectively, compared to those under the CK. Additionally, DCAS ventilation effectively reduced the incidence of tipburn in lettuce. The above results indicate that the DCAS can be considered as an effective system for improving canopy microenvironment, plant growth and reducing tipburn occurrence.

Key words: *plant factory, airflow, CFD, canopy microenvironment, plant quality.*

INTRODUCTION

As plant factories continue to expand in scale and increase cultivation layers, the limitations of traditional ventilation systems focusing on overall environmental control have become increasingly apparent, failing to meet the precise airflow regulation requirements in plant cultivation spaces (Zhang et al., 2024). In conventional ventilation systems, low airflow velocities within cultivation areas, including stagnant airflow zones, lead to uneven distribution of environmental factors such as temperature, humidity, and CO_2 concentration. This results in stunted plant growth and physiological disorders like tipburn (Sago, 2016). Furthermore, insufficient airflow velocity adversely affects exchanges of energy and matter between plant and environment, particularly in CO_2 and H_2O exchange processes, consequently impairing photosynthetic and transpiration rates and hindering plant development (Baek et al., 2015). Therefore, improving ventilation methods and optimizing airflow velocity distribution within plant cultivation spaces currently represent the most effective solutions to these challenges (Zhang et al., 2022).

Airflow is a complex environmental factor that exerts diverse effects on plants, depending on its velocity, duration, and penetration depth into the plant canopy. Sufficient airflow velocity can

influence plant development, morphology, and physiological functions. Studies have shown that airflow exerts mechanical stimulation on plants, resulting in shorter but thicker stems and enhanced lodging resistance (Gardiner et al., 2016). Thus, airflow can induce biological adjustments in plant height and elongation. Additionally, uneven airflow distribution may lead to asymmetric plant growth, causing plants to bend away from the direction of stronger airflow. Therefore, the impact of airflow on plant growth is dual-sided—appropriate airflow velocity can promote plant growth and development while improving crop quality. Kitaya, (2005) reported that increasing the horizontal air velocity from 0.005 to 0.8 m s^{-1} enhanced the cucumber transpiration rate by 2.1 times. Previous studies have shown that the optimal airflow velocity range for plant canopies is between 0.3 and 1.0 m s^{-1}, within this range, plants exhibit significantly improved photosynthetic and transpiration rates while also experiencing the lowest probability of disease occurrence.

The objective of this study was to design a double aeration cultivation system (DCAS). CFD software was utilized to simulate the airflow distribution within the cultivation space under different intake air velocities. The optimal intake air velocity was determined through airflow uniformity. Practical plant cultivation experiments were conducted with the above optimal intake air velocity. Finally, the optimal intake air velocity was determined according to the growth of lettuce.

MATERIALS AND METHODS

2.1. Numerical simulation

2.1.1. The model geometry and grid generation

Based on the size of the cultivation shelves, the DCAS was designed with dimensions (length × width × height) of 1.0 m × 0.3 m × 0.12 m. Two DCASs could be placed on each layer. The structure of DCAS was shown in Figure 1.

According to the height of lettuce at maturity, the plant canopy height was set at 0.15 m. Considering the distance between the LED panel and the cultivation board, the air layer was set at 0.25 m. In the Geometry module of Workbench, the DCAS model was established, with the northeast corner of the model as the origin point O (0, 0, 0). The positive east direction was defined as the X-axis, the vertical upward direction as the Y-axis, and the positive north direction as the Z-axis.

The DCAS model (Figure 2), including the plant canopy, air layer, ventilation ducts, and LED heat source, was constructed in the Geometry module of Workbench. The completed DCAS model was then imported into the Mesh module, where the Proximity and Curvature method was applied for grid generation (Figure 3). To improve simulation accuracy, the mesh near the walls of the ventilation ducts was refined. Two different angles generated 254,078 and 257,154 grid cells, respectively. The mesh quality was evaluated using skewness, with an average skewness of 0.21 and a maximum skewness of 0.72, meeting the required standards.

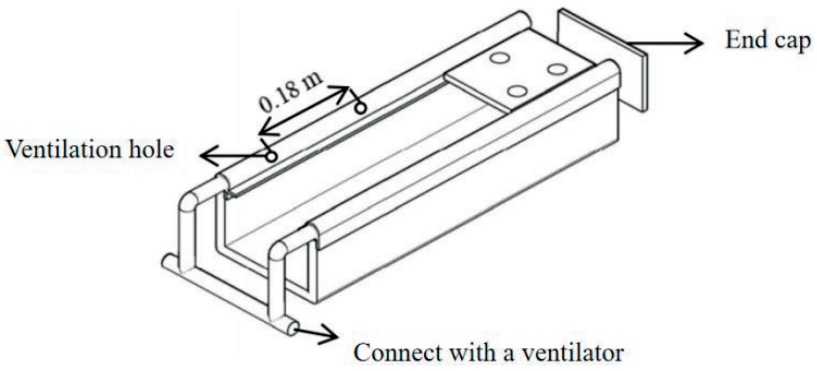


Figure 1 Double channel aeration cultivation system

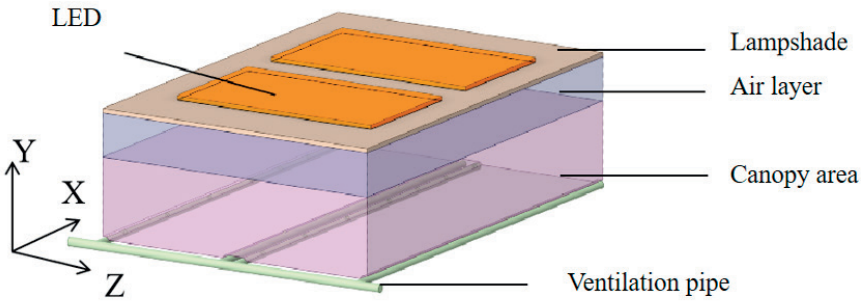


Figure 2. The CFD model of double aeration cultivation tank

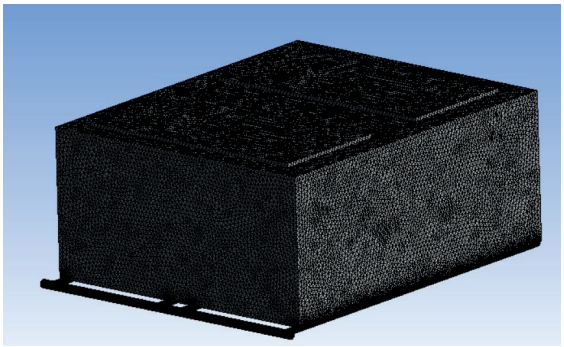


Figure 3. The grid of CFD model

2.1.2. The numerical approach

In this study, the distribution of airflow in the crop canopy was mainly affected by the structure of the cultivation tank and the intake air velocity. The fluid flow in the problem domain was assumed to be a steady-state, incompressible, and three-dimensional turbulent. The numerical calculation of airflow can be seen as a mathematical formula for the conservation law of fluid mechanics. In this model, the momentum equation was used, and turbulence model was selected to solve for the turbulent kinetic energy and the viscous dissipation rate of turbulent energy. The governing equations included the continuity equation, the momentum equation, the turbulent kinetic energy equation, and the dissipation rate equation, these equations can be represented by the following general equations (Versteeg, 1995):

$$\frac{\partial \rho}{\partial \tau} + \frac{\partial}{\partial X_i}(\rho u_j) = 0 \qquad (2\text{-}1)$$

$$\frac{\partial}{\partial t}(\rho u_i) + \frac{\partial}{\partial X_j}(\rho u_j u_i) = \frac{\partial}{\partial X_i}[-P\delta_{ij} + \mu(\frac{\partial u_i}{\partial X_j} + \frac{\partial u_j}{\partial X_i})] + \rho g_i \qquad (2\text{-}2)$$

$$\frac{\partial}{\partial t}(\rho C_a T) + \frac{\partial}{\partial X_j}(\lambda \frac{\partial T}{\partial X_j}) = S_T \qquad (2\text{-}3)$$

2.1.3. Boundary conditions

In this experiment, the DCAS model was simplified to reduce computational load, retaining only the ventilation ducts, plant canopy, the air layer above the canopy, and the LED heat source. The plant canopy was modeled as a porous medium, with the viscous resistance coefficient and inertial resistance coefficient set to 25 and 1.3, respectively (Zhang et al., 2019). The air layer was defined as an air medium, and an LED heat source model was embedded above it. The lamp cover (glass) was treated as a

conductive material, while the reactor (aluminum) was defined as the heat source, with a volumetric heat generation rate of 34,166 $W \cdot m^{-3}$ (Zhang et al., 2021). The duct inlet was set as a velocity inlet, with airflow velocities of 5, 6, 7, and 8 $m \cdot s^{-1}$, respectively. The outlets around the canopy were defined as pressure outlets (0 Pa). The thermal physical properties of the materials used in the simulation are listed in Table 1 (Wu et al., 2021).

Table 1. Thermal physical parameters of different materials in CFD model

Material	Density ($kg\ m^3$)	Dynamic viscosity ($kg\ m^{-1}\ s^{-1}$)	Specific heat ($J\ kg^{-1}\ K^{-1}$)	Thermal expansion coefficient (K^{-1})	Thermal conductivity ($W\ m^{-1}\ K^{-1}$)
Air	1.225	1.7894×10^{-5}	1006.43	3.43×10^{-3}	2.42×10^{-2}
Canopy	998.2	/	4182	/	0.6
Glass	2500	/	670	/	0.74
Aluminum	2719	/	871	/	202.4

2.2. Cultivation experiment setup

2.2.1. Plant preparation

Lettuce seeds (*Lactuca sativa* cv.'Butterhead') were germinated in sponge cubes and incubated for 2 days in a growth chamber in the dark at an air temperature of 23.5°C. On day 3, seedlings were transferred to a plant factory with PPFD of 150 $\mu mol\ m^{-2}s^{-1}$ by using fluorescent lamps (ZPDT812WW-75, ZSP Technology Co, China). On day 15, uniformed-size seedlings were selected and transferred to the greenhouse with a planting density of 28 plants/m^2 and a spacing of 0.15 m. Yamazaki lettuce nutrient solution was selected as the nutrient solution.

2.2.2. Plant measurements

On day 28 after transplanting, fresh and dry weights of shoots and roots, number of leaves per plant and total leaf area were measured. The fresh weights of shoots and roots were measured using an electronic scale (GL6202-1SCN, Sartoriu, Germany). Shoots and roots were dried in an oven at 80 °C for 72 h. Total leaf area was measured using a leaf area meter (Li-3100C, LI-COR, Lincoln, NE, USA). Ten plants were chosen randomly from each treatment for measurement.

2.2.3. Environmental parameters measurements

On day 28 days after transplanting, the air velocity in plant canopy were measured by an infrared hot-wire anemometer (Climomaster6501-BG, KANOMAX, JAPAN) along the cultivation tank. Sensors (LR5001, HIOKI, JAPAN) were used to record the air temperature, relative humidity within the plant canopy for 28 days.

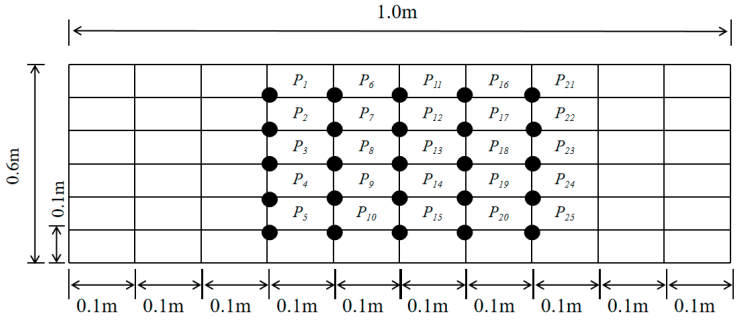


Figure 4. The air velocity measured points in plant canopy

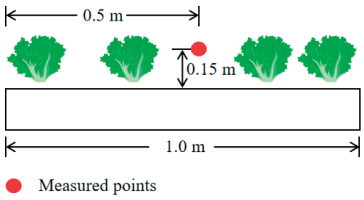


Figure 5. The air temperature and relative humidity measured points within plant canopy

2.2.4. Experiment setup

The experiment comprised four treatments: the control group utilized the existing ventilation system of the artificial light plant factory, generating an average canopy airflow velocity of 0.2 m s^{-1}.

The other three treatments employed the Double Channel Aeration Cultivation System (DCAS) to provide distinct airflow velocities: 0.6 (T_1), 0.9 (T_2), and 1.2 m s^{-1} (T_3).

These were achieved by connecting a ventilator (JYF-75pqs, Shenzhen) to the duct inlet and operating it at 50%, 75%, and 100% of its rated power capacity respectively.

2.3. Statistical analysis

Statistical analysis was carried out using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) statistical software. Separation of means was performed using a multiple range test of least significant difference (LSD) at $p \leq 0.05$.

RESULTS

3.1. Model validation

Under a ventilation angle of 45°, the simulated and measured wind speed values at 25 measurement points were compared (Figure 6).

When the inlet velocities were set at 5, 6, 7, and 8 $m \cdot s^{-1}$, the average relative errors between the simulated and measured values were 7.5%, 4.4%, 7.7%, and 3.7%, respectively.

Overall, the simulated results showed good agreement with the measured data, exhibiting similar trends.

Thus, the model's accuracy meets the required standards and can be reliably used for data analysis.

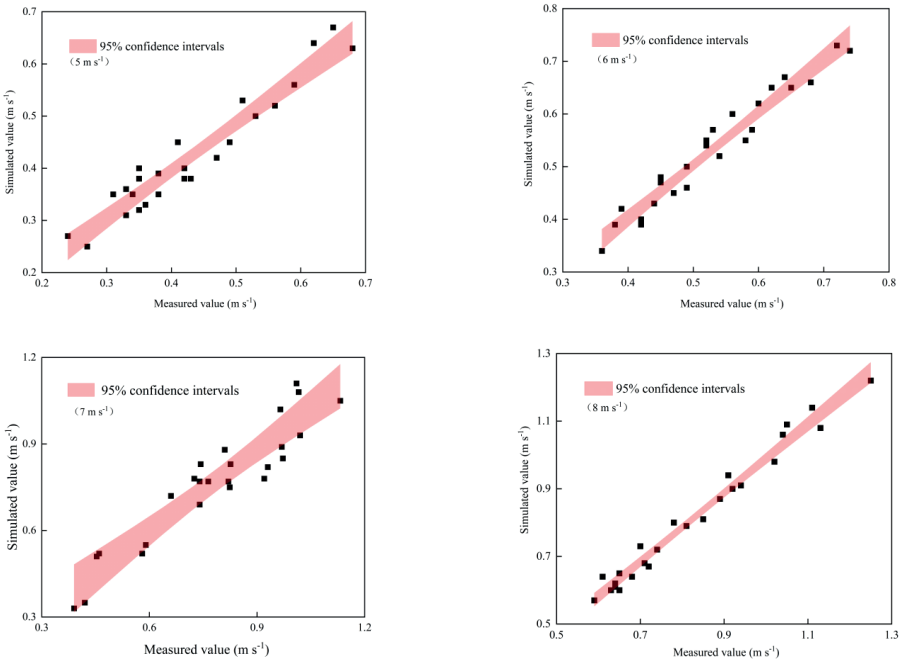


Figure 6. Comparison of simulated and measured values of airflow velocity in plant canopy under different inlet air velocities

3.2. The airflow distribution within plant canopy under ventilation angle of 45°

The analysis was conducted on the Z=0.3 m plane. In this experiment, the coefficient of variation (CV) was employed to evaluate airflow uniformity, where CV is defined as the ratio of standard deviation to mean value. A higher CV indicates poorer airflow uniformity. The airflow formed distinct jets upon entering the canopy, with subsequent velocity gradually attenuating until exiting the canopy. When the inlet velocity increased from 5 to 8 m s^{-1}, the proportion of favorable zone in the plant canopy rose from 64.8% to 71.1%, while the stagnant zone decreased from 35.2% to 28.9%, and the average airflow velocity within the canopy increased from 0.35 to 0.71 m s^{-1} (Table 2). However, this increase in inlet velocity resulted in reduced airflow uniformity throughout the canopy.

Table 2. The proportion of canopy airflow in each area of different intake air velocities under ventilation hole angle of 45°

Intake velocity (m s^{-1})	Volume proportion (%)			CV (%)	AVG (m s^{-1})
	V<0.3 m s^{-1}	0.3 m s^{-1}≤V≤1 m s^{-1}	V>1 m s^{-1}		
5	35.2	64.8	0	57	0.35
6	33.1	66.9	0	60	0.49
7	31.5	68.5	0	64	0.6
8	28.9	71.1	0	70	0.71

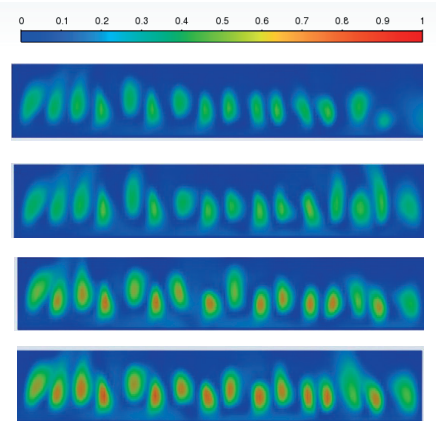


Figure 7. Airflow distribution of canopy in the plane of Z=0.3 m under ventilation hole angle of 45°

3.3. The airflow distribution within plant canopy under ventilation angle of 60°

As the inlet air velocity increased from 5 m s^{-1} to 8 m s^{-1}, the proportion of favorable zones in the plant canopy increased from 51.4% to 58.4%, while the stagnant zones decreased correspondingly from 48.6% to 41.6% (Table 3). Concurrently, the average airflow velocity within the plant canopy rose from 0.31 m s^{-1} to 0.64 m s^{-1}. However, this increase in inlet velocity was accompanied by a reduction in airflow uniformity throughout the canopy.

Table 3. The proportion of canopy airflow in each area of different intake air velocities under ventilation hole angle of 60°

Intake velocity (m s^{-1})	Volume proportion (%)			CV (%)	AVG (m s^{-1})
	V<0.3 m s^{-1}	0.3 m s^{-1}≤V≤1 m s^{-1}	V>1 m s^{-1}		
5	48.6	51.4	0	64	0.31
6	46.5	53.5	0	69	0.42
7	43.2	56.8	0	72	0.55
8	41.6	58.4	0	77	0.64

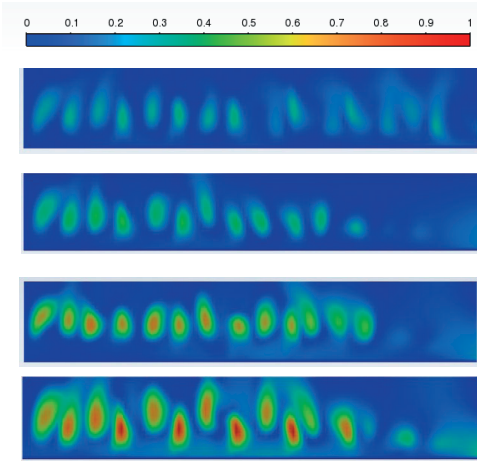


Figure 8. Airflow distribution of canopy in the plane of Z=0.3 m under ventilation hole angle of 60°

3.4. Comparative analysis of airflow distribution under different ventilation angles

The comparison of airflow velocity distribution contours in the canopy plane clearly demonstrates that the 45° ventilation angle configuration produces more uniform airflow from each vent while maintaining superior performance characteristics at equivalent inlet velocities, including a higher proportion of favorable airflow zones, greater average canopy airflow velocity, and lower coefficient of variation compared to other configurations. Both tested vent angles showed increasing proportions of favorable zones with higher inlet velocities without generating any high-velocity zones, though accompanied by reduced airflow uniformity, with the 45° configuration consistently exhibiting better overall performance.

3.5. The environmental parameters as affected by different canopy air velocities

During the experimental period, the temperature in the plant factory ranged from 21-26°C.

As shown in Figure 8, both the relative humidity at the plant canopy and the temperature during the light period varied over time.

The results demonstrated that increasing airflow velocity through the canopy led to corresponding decreases in both relative humidity and light-period temperature.

Compared to the control group, the three experimental treatments showed average relative humidity reductions of 1.59%, 3.7%, and 6.6%, respectively, along with average light-period temperature decreases of 1.7°C, 2.2°C, and 2.8°C.

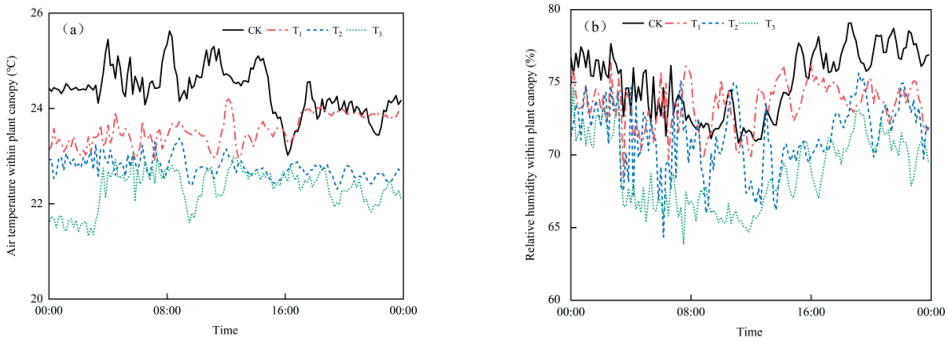


Figure 9. The time course of relative humidity (a) and air temperature (b) within plant canopy on day 21 after transplanting of different treatments

3.6. The lettuce growth and tipburn occurrence as affected by different canopy air velocities

The growth parameters of lettuce are presented in Table 4. As the canopy airflow velocity increased, the fresh weight of lettuce shoots initially increased and then decreased, reaching its maximum value of 56.66 g at a canopy airflow velocity of 0.9 m s^{-1}. The dry weight of shoots followed a similar trend, peaking at 3.14 g under the same airflow velocity. Different canopy airflow velocities showed no significant effect on root growth. Notably, lettuce plants grown under DCAS exhibited no leaf tipburn while the control group displayed this disorder. The number of leaves remained unaffected by variations in canopy airflow velocity.

Table 4. The growth and tipburn occurrence of lettuce under different treatments

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Tipburn occurrence(%)	Total leaf number
CK	49.8±1.9c	2.5±0.2b	10.6±0.8a	0.63±0. 1a	20.9±4.5	21±1.0a
T_1	55.2±2.2ab	2.7±0.3ab	9.7±0.6a	0.52±0.1a	0	20±1.0a
T_2	56.7±4.1a	3.1±0.3a	10.2±1.2a	0.57±0.1a	0	21±1.0a
T_3	53.9±4.5b	2.7±0.4ab	9.4±1.5a	0.55±0.1a	0	21±1.0a

DISCUSSIONS

Enhancing both crop yield and quality represents two primary objectives in protected agriculture. In this study, lettuce shoot biomass (both fresh and dry weight) initially increased with elevated canopy airflow velocity, but decreased when velocity exceeded 1.2 m s^{-1}. The yield improvement can be attributed to reduced leaf boundary layer resistance, which enhances CO_2 assimilation and H_2O diffusion (Okayama et al., 2008). However, excessive airflow velocities may induce over suppressed transpiration rates, leading to plant water deficit.

Substantial evidence indicates that insufficient airflow velocities (<0.3 m s^{-1}) compromise photosynthetic efficiency by restricting CO_2 uptake while simultaneously impairing transpiration-driven nutrient transport, ultimately degrading crop quality. Optimal lettuce development typically occurs within 0.3-1.0 m s^{-1} (Lee et al., 2000), with velocities exceeding 0.3 m s^{-1} demonstrably benefiting both photosynthesis and transpiration (Kitaya et al., 2003).

In leafy vegetable production, proper airflow management plays another critical role in mitigating tipburn which is a calcium-related physiological disorder particularly prevalent in lettuce cultivation characterized by necrotic leaf margins and subsequent browning, tipburn incidence correlates positively with growth rate (Borkowski et al., 2016). Under rapid growth conditions, calcium demand for new tissue development often outstrips supply due to limited transpiration-driven calcium transport (White et al., 2003). Conventional facilities with suboptimal airflow (<0.5 m s^{-1}) exacerbate this condition by thickening boundary layers and suppressing transpiration. Notably, the DCAS completely prevented tipburn occurrence by delivering consisten airflow directly to the canopy, whereas conventional ventilation failed to penetrate the canopy effectively, resulting in tipburn.

CONCLUSIONS

A double channel aeration cultivation system (DCAS) was designed and created to improve the airflow distribution within lettuce plant canopy, as well as the complexity of adding ventilation equipment. CFD software was used to simulate the airflow distribution and uniformity within the plant canopy under different ventilation parameters. To validate the practical application of the cultivation troughs, a cultivation experiment was conducted in the plant factory. The effects of DCAS ventilation and conventional ventilation modes on lettuce growth and microenvironment within plant canopy under different airflow velocities were

tested and analyzed. The main conclusions are as follows.

1) In the CFD simulation, two ventilation angles were designed. By comparing airflow distribution cloud diagrams and airflow field analyses, the optimal ventilation parameters were determined to be a 45° ventilation angle and an airflow velocity of 8 m s^{-1}.

2) In the cultivation experiment, lettuce grown under conventional ventilation exhibited slow growth and tipburn. In contrast, lettuce grown under the DCAS showed no tipburn, increased yield, and lower canopy temperature and relative humidity compared to the conventional ventilation mode.

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IN VITRO MULTIPLICATION AND ACCLIMATIZATION OF THE ROYAL FERN, *OSMUNDA REGALIS* L.

Florentina ALDEA, Florența-Elena HELEPCIUC, Elena Monica MITOI

Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independenței, District 6, Bucharest, Romania

Corresponding author email: florentaelenah@gmail.com

Abstract

Osmunda regalis L. *(royal fern) is a species with special ornamental values that can reach 2.5 m in height. The species is extinct in Romania and threatened in several European countries, it prefers humid habitats with spores having a short viability period. . Our studies focused on the introduction of this species in in vitro culture, in order to propagate the sporophytes and subsequent acclimatization. The spores were sterilized and inoculated on the nutrient medium MS ½, the differentiation of the protalli being achieved after approximately 3 weeks from the initiation of the culture. Sexual multiplication was achieved by differentiating sporophytes in gametophytic cultures on nutrient depleted medium MS½ without ammonium nitrate and vitamins. Later, clonal multiplication was achieved by detaching the sporophytes and subcultivation on the same type of medium. Acclimatization was carried out on seven substrate variants, with different pH, composition and granulation, the best results being obtained on the substrate which contained 80% peat and 20% humus. Through this technique, it was possible to obtain viable sporophytes, also, rooting and acclimatization at the greenhouse level for horticultural exploitation or the repopulation of suitable habitats.*

Key words*: Osmunda regalis, fern, gametophyte, sporophyte.*

INTRODUCTION

Osmunda regalis L., royal fern, is a species of Pteridophyta from Osmundaceae family, one of the oldest families of ferns, whose fossils date back over 200 million years to the Permian (Smith et al., 2006; Hewitson, 1962). The name reflects its imposing and elegant appearance. It is a perennial, isospore, with a vigorous, strongly branched oblique rhizome (Săvulescu, 1952). The fronds are double-pinnate, arranged in a dense, majestic crown that can reach 2.5 m in height (https://www.rhs.org.uk/plants/trials-awards).

The species *O. regalis* was valorised in horticulture and landscaping due to its appearance; its spectacular foliage in autumn acquires shades of red and brown, creating a visual pleasant aspect. The Royal Horticultural Society awarded the species *Osmunda regalis* by giving the prestigious Award of Garden Merit for outstanding horticultural characteristics (https://www.rhs.org.uk/plants/trials-awards).

The rhizome of this fern has been used as a substrate for growing orchids and epiphytes, and in Russia and Europe, it is used for horticultural purposes (Matchutadze, 2014; https://www.missouribotanicalgarden.org; https://herbaria.plants.ox.ac.uk)

Although not recorded in official pharmacopeias, this species has a long history of use in traditional medicine. The roots and leaves of the *O. regalis* species have been used to treat jaundice, rheumatic pains, bone fractures, wounds (Matchutadze, 2014). In some regions of Spain, it is rare and is classified as endangered due to its harvesting for medicinal purposes. A macerate with white wine (antojil wine) is prepared from the middle part of the rhizome and is used as a remedy for digestive disorders (Molina et al., 2009).

The decrease in precipitation and the expansion of drainages contribute to the reduction of habitat and essential resources for this species, increasing the risk of extinction.

According to the IUCN assessments, 2014 the species *O. regalis* is critically endangered in Belarus, Bulgaria, Croatia, Hungary, Iran and Luxembourg, vulnerable in Germany, Switzerland and Poland (Gdula et al. 2014) and rare in Norway (Birks et al., 1991).

In Romania, *O. regalis* is mentioned as an extinct species in two Red Lists (Boșcaiu et al., 1994; Dihoru & Dihoru., 1994).
Under typical storage conditions, the spores of the species *O. regalis* showed reduced viability (Stokey, 1951; Windham et al. 1986).
O. regalis has a long life cycle, of almost two years, from spore sowing to the first production of sporophytes and spores (Klekowski, 1967).
In the current context of anthropogenic changes and pressures on biodiversity, taking into account the specific ecological characteristics of the species, which includes a preference for humid environments and a long-life cycle, *in vitro* micropropagation and the acclimatization process represent an alternative for the revitalization of the *O. regalis* species. Recent studies on extracts obtained from this plant have identified some of the bioactive compounds responsible for the beneficial effects (Mitoi et al., 2024) and analysed the impact of osmunda extracts on cancer cells, demonstrating their potential use as anticancer remedies (Schmidt et al., 2017; Carpinteyro Diaz et al., 2024).
The distribution area of the species includes temperate and tropical regions. It prefers moist and shady habitats, growing in peatlands, swamps, lake shores, and forests, on moist and peaty mineral soils (Birks et al., 1991; Matchutadze, 2014).
The aim of this study was to identify an efficient method of multiplication and acclimatization using *in vitro* culture technique for the species *O. regalis*. The objective was to obtain a large number of sporophytes through sexual reproduction *in vitro* and vegetative multiplication of the obtained sporophytes, as well as to determine an adequate substrate that would meet the specific nutritional requirements of this pteridophyte, to ensure a high survival rate upon acclimatization to greenhouse conditions.

MATERIALS AND METHODS

Plant material

The biological material used to initiate *in vitro* culture was represented by spores harvested from plants grown in the Dimitrie Brândză Botanical Garden from Bucharest. The spores were preserved at 4°C until use.

Obtaining the gametophytic cultures from spores

For sterilization, the spores were introduced in filter paper envelopes and immersed in a 3% H_2O_2 solution or in a mixture of 6% $CaCl_2O_2$ with Tween 20 for 7-10 minutes, followed by washing three times with sterile distilled water.
The spores were inoculated in Petri dishes on MS basal medium (Murashige and Skoog, 1962) with half concentrations of micro and macroelements, supplemented with vitamins B5 (Gamborg et al., 1968) and 30 g/L sucrose, at an adjusted pH of 5.8 (MS ½).
The culture vessels were maintained in growth chambers at a temperature of 22-24 ±2°C, with a photoperiod of 16 hours at a fluorescent intensity of 40.5 µmol/m^2s.
Gametophytes obtained from spore germination were subcutivated at each 2-month, on the same type of nutrient medium MS ½.

Obtaining sporophytes from gametophytes by sexual reproduction

For sporophytes differentiation from gametophytes, 1 g of mature gametophyte was transferred to Erlenmeyer flask on MS ½ solid nutrient medium with or without ammonium nitrate and vitamins (Makovski et al., 2016). The three experimental variants were:

1) MS ½ solid;
2) MS ½ solid without ammonium nitrate and vitamins- MFAV;
3) MFAV, to which 1 ml of same liquid medium was added to the gametophytes surface.

Sporophytes cultures were inoculated in 250 ml Erlenmeyer flasks containing 100 ml of nutrient medium and incubated under the same growth conditions mentioned above. For each nutrient medium variant, 6 repetitions were performed. The number of sporophytes was evaluated at 4, 5 and 6 months after inoculation.

Evaluation of sporophyte leaves and roots growth

Sprophytes obtained on MFAV were detached from the gametophyte and inoculated on four culture medium variants:

1) MS ½ solid;
2) MFAV solid;

3) MSR- MS nutrient medium supplemented with 1 mg/l benzylaminopurine (BAP), 1 mg/l kinetin (K), 1 mg/l 1- naphthalenacetic acid (ANA);
4) E7- MS nutrient medium supplemented with 1.8 mg/l indolilacetic acid (AIA), 0.02 mg/l K.

The cultures were incubated in growth chambers at a temperature of 22-24±2°C, with a photoperiod of 16 hours. After 3 months from inoculation, the sporophytes were weighed and measured, by registration of the following morphometric parameters: sporophyte growth rate (SGR), leaf number (LN), the largest leaf length (LLL), the smallest leaf length (SLL), rootlets number (RN), the longest rootlet length (LRL), the smallest rootlet length (SRL).

Multiplication of sporophytes

Vegetative multiplication was achieved by sectioning the sporophytes in half at the rhizome level and inoculating them on fresh MFAV medium. The sporophyte cultures were grown in 100 and 250 ml Erlenmeyer flasks or vessels with 50 and 100 ml medium under the same controlled conditions mentioned above.

One year after the experiment started, the following gravimetric and morphometric parameters were determined: sporophyte growth rate (SGR), leaf number (LN), the largest leaf length (LLL), the smallest leaf length (SLL), the longest rootlet length (LRL).

Obtaining gametophyte from leaves and rootlets fragments

Leaves and rootlets from juvenile sporophytes were detached or sectioned using a sterile scalpel and cultured on MFAV medium. After 20 days from inoculation, the gametophyte began to develop.

Acclimatization of sporophytes to soil in pots

In a first experiment for acclimatization, 1-year-old sporophytes were used and three substrates variants were tested (Table 1):

- substrate for indoor plants (S1);
- substrate for sowing and propagation with 80% peat and 20% humus (S2);
- -substrate for ornamental shrubs with bark enriched with basic nutrients and trace elements B, Cu, Mn, Mo, Zn, Fe (S3). Additionally, for this experiment the sterilized variants of these substrates were used, SS1, SS2 and SS3.

The plant growth process was monitored over a period of one year. The assessments were carried out in four stages: at the beginning of acclimatization, at one month, three months and at one year of potting plants. The analyzed parameters included: sporophyte growth rate (SGR), leaf number (LN), the largest leaf length (LLL), the smallest leaf length (SLL), root length (RL), radicular volume (RV), survival percent of sporophytes (SP).

In the second acclimatization experiment, four other four types of substrates were tested:

- aquatic plants substrate with peat, quality compost, sand, clay (S4);
- acidophilous plants substrate with horticultural peat (S5);
- cacti substrate (S6);
- natural peat substrate (S7).

Also, the sterilized variants SS4, SS5, SS6 and SS7 were used.

Plant growth was monitored one month after the sporophytes transfer to the pots. The assessments included the same biometric parameters analysed as in the first acclimatization experiment.

All types of substrate except variant 7 were purchased from Agro CS Romania SRL. In both experiments, both sterile (SS1, SS2, SS3, SS4, SS5, SS6 and SS7) and non-sterile (S1, S2, S3, S4, S5, S5 and S7) substrates were tested, differentiated according to pH, composition and conductivity (Table 1).

Table 1. Some physicochemical parameters of the used substrates.

Substrate	pH	N (mg/l)	P_2O_5 (mg/l)	K_2O (mg/l)	Conductivity ($mS.cm^{-1}$)
S1	4.7	150-200	100-200	200-300	0.8
S2	5.5	300	100	300	0.8
S3	5.5	50-250	80-250	100-300	0,8
S4	6.7	100-200	80-200	100-300	0.8
S5	4.5				0,5
S6	4.6	20-150	30-150	40-200	0,5
S7	4.2				

Planting was carried out in plastic seedling pots using the same amount of substrate. Each experimental variant had 6 repetitions.

Acclimatization was carried out under natural lighting conditions, at a temperature of 22 °C, and humidity of 40-45%. To ensure a humidity level similar to that of *in vitro* cultivation conditions (approximately 80%), the plants were covered with glass pots. Adaptation to

greenhouse environmental conditions was achieved gradually. The plants were uncovered daily for 10 minutes, with exposure to ambient atmospheric conditions gradually increasing until complete acclimatization was achieved.
The gravimetric and morphometric parameters used for all the experiments were obtained as follows:

- growth rate = final weight – initial weight/initial weight;
- roots and leaves length were measured with a ruller and calculated as difference between initial and final value;
- total root volume, determined by the volumetric method.

All gravimetric measurements were made on the adwag AS 220/C/2 balance (Radwag, Radom, Poland)

RESULTS AND DISCUSSIONS

The life cycle of *O. regalis* fern is characterized by alternation of generations, which includes a haploid gametophytic phase, involved in the production of gametes, and a diploid sporophytic phase responsible for sporogenesis. The sporophytic phase is dominant, characterized by morpho-functionally differentiated roots, rhizomes, and fronds, including specialized leaves (sporophylls) that contain sporangia, where meiosis and the formation of haploid spores occur.
Under our experimental conditions of *in vitro* culture, involving aseptic spores germination, prothalli were differentiated approximately three weeks after inoculation (Figure 1a) . The obtained gametophytic culture was subsequently cultured for 2 months on MS½ medium (Figure 1b). This gametophytic culture was multiplied and maintained in long-term *in vitro* conditions for several years.

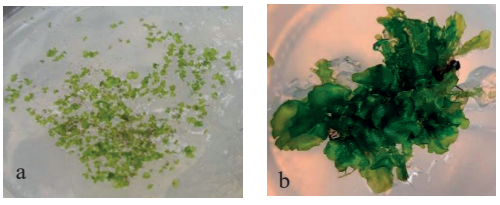


Figure 1. Gametophytes cultures of *O. regalis*: a- primary protalli culture and b- gametophyte culture

Evaluation of the gametophyte growth rate
Initially, the growth of the gametophyte culture was carried out on half strength MS medium (MS½), because our previous studies on other fern species showed that this nutritive medium proved to be the most advantageous (Aldea et al., 2016). Subsequently, a better gametophyte development was observed by using the solid MS½ medium without NH_4NO_3 and vitamins (MFAV) in order to obtain sporophyte differentiation. Evaluation of the growth rates showed a higher gametophyte biomass accumulation on the MFAV medium comparedto the MS½ medium (Fig. 2).

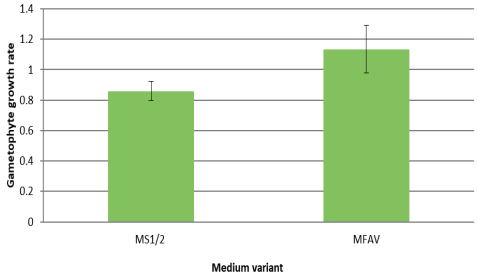


Figure 2. The growth rate of *O. regalis in vitro* gametophyte cultures

O. regalis, like most ferns, is a plant adapted to grow in poor nutrient media. Fernandez et al., 1997 and 1999 demonstrated the autotrophy of the gametophyte of these species, which can grow on sucrose-free media. The same authors observed, similar to our results, a reduction in gametophyte growth when ammonium and sucrose were added to the Knop medium (Knop, 1865).

Differentiation of sporophytes from the gametophyte culture by sexual reproduction
Sporophytes began to differentiate on gametophyte culture after three months of cultivation on the MFAV medium (Figure 3) . A layer of liquid medium applied on the gametophyte surface had a positive effect on sporophyte differentiation.
After four months of subcultivation, an exponential increase in the number of sporophytes was observed in cultures supplemented with liquid medium (Figure 4). This increase was maintained after six months, the number of sporophytes being almost double

compared to those without liquid medium applied to the gametophyte surface.



Figure 3. The sporophyte differentiated on gametophyte culture in *O. regalis*

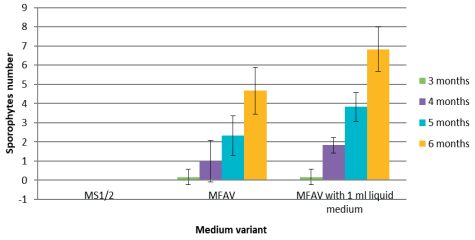


Figure 4. The number of sporophytes differentiated on different culture media

These results suggest that the presence of fluid on the gametophyte surface contributed to the fertilization process, by facilitating the mixing of gametes from antherozoids with archegonia differentiated on the gametophyte surface (Figure 5). Our cultures were unable to differentiate sporophytes on solid MS ½ medium during the cultivation period.

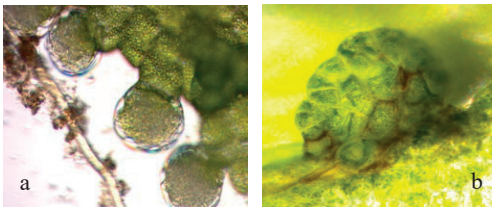


Figure 5. Antheridia (a) and archegonia (b) differentiated in gametophytes culture

However, other authors have shown that gametophytes produced archegonia and antheridia in large numbers on MS½ and MS¼ media (Markowski et al. 2016), these cultures being able to produce sporophytes after 15 months. The authors confirmed, also, sexual reproduction in this species through flow cytometry studies.

In our previous studies, cultivation on MS½ medium and the gametophyte homogenization technique by mortaring provided favorable results for other tested species such as *Polypodium vulgare*, *Asplenium trichomanes* and *Athyrium filix-femina* (Aldea, 2016), but for the species *O. regalis* this technique did not have positive results (Mitoi et al., 2024).

The formation of sporophytes on the surface of the gametophyte in *O. regalis* was described for the first time, in gametophyte cultures on Knopp medium after 6 months of cultivation at a low rate of 2.5 sporophytes/g fresh weight gametophyte (Fernandez et al., 1997). This medium does not contain NH_4NO_3, but contains 625 mg/l KNO_3. Later, the same authors reported an increase in sporophyte differentiation to approximately 20 sporophytes/g fresh weight, when the gametophyte was cultured in the absence of nutrients, on a medium containing only distilled water and 0.7% Difco Bacto-agar (Fernandez et al., 1999). Other authors obtained sporophyte differentiation in 15-20% of gametophytes on MS medium modified by replacing the macroelements with those of H&A medium (Hoagland and Aron, 1950) after three months of subcultivation (Morini, 2000). The best results were reported by Makowscki et al., (2016) on MS1/8 medium without NH_4NO_3 and without vitamins after 4 months of cultivation. These authors found, by using Knopp medium, diluted MS media, but also by eliminating ammonium and vitamins from the MS medium, that both the dilution of the culture medium and the absence of ammonium were important for achieving the fertilization process.

Other studies suggested that both the nitrogen source (NH_4NO_3 vs KNO_3) and its level may regulate sporophyte formation in sexual reproduction in other species such as *Equisetum arvense* and *Adiantum capitum-venere* (Kuriyama et al., 1992 and 2004).

All these studies showed that nitrogen, which is an important resource for heterotrophic plants, had negative effects on sporophyte formation in *O. regalis*. Not only the elimination of the

supplementary 1.600 g/l NH_4NO_3 from the composition of the MS medium had beneficial effects on the formation of sporophytes, but also the dilution of this medium, which also contains KNO_3 in a concentration of 1.900 mg/l had a beneficial effect. By diluting the MS medium to MS1/8, the remaining KNO_3 concentration was approximately 237.5 mg/L, making it the best option.

The difficulty of sporophyte formation in this species on nitrates and nitrites enriched media may also represent an explanation for its disappearance from characteristic areas. Climate change, as well as agricultural practices of large-scale use of nitrogen-based fertilizers, have probably contributed to the restriction of the spread of this species, through the impossibility of sexual sporophyte differentiation in this species at higher nitrogen concentrations.

Evaluation of sporophytes development

Four types of culture media were tested for evaluation of sporophytes roots and leaves growth. The best results were also obtained on the MFAV medium, the sporophyte growth rate being 1.65±0.25 at 3 months. The media supplemented with hormones had no beneficial effects on sporophyte development, both at the root and leaf level, except for the number of differentiated leaves in the MSR variant (Table 2). Following the measurements performed, it was observed that sporophytes can also develop on the MS½ medium, although the analysed morphometric parameters were significantly lower than MFAV, but registering higher values compared to the other experimental variants with hormones (MSR and E7).

Table 2. Gravimetrical and leaf morphometrical parameters of sporophyte development on different culture media at three months after inoculation

Nutritive medium variants	RN (cm)	LRL (cm)	SRL (cm)
MS½	1.5±1.04	0.21±0.07	0.18±0.16
MFAV	1.83±0.75	0.28±0.09	0.28±0.05
E7	0.16±0.40	0.01±0.04	0.01±0.04
MSR	0.16±0.40	0.01±0.04	0.01±0.04

These results are also supported by previous studies in *O. regalis* which showed that supplementation with hormones such as benzyl adenine (BA), gibberellic acid (GA3) and naphthaleneacetic acid (NAA) led to vitrification of sporophyte tissues (Morini, 2000). Subsequently, in this study sporophytes were grown on H&A medium without hormones which favoured vigorous development of leaves and roots, but differentiation of new leaves or roots was rarely observed. In contrast, our results showed a leaf differentiation rate of 1.5±0.83/3 months and 6.33±2.5/12 months of cultivation, while root differentiation was 1.83±0.75/3 months and 6.83±1.16/12 months of cultivation on MSFAV medium (Table 3).

Table 3. Roots morphometrical parameters of sporophyte development on different culture media at three months after inoculation

Nutritive medium variants	SGR	LN	LLL (cm)	SLL (cm)
MS½	1.04±0.21	0.83±0.40	0.31±0.13	0.35±0.25
MFAV	1.65±0.25	1.5±0.83	0.6±0.14	0.43±0.12
E7	0.11±0.10	-0.16±0.75	0.1±0.06	0.08±0.07
MSR	0.14±0.11	1.16±1.32	0.2±0.12	0.11±0.09

Multiplication by sporophyte division at the rhizome level

Our experiment showed that the development of sporophytes were not significant affected by the rhizome sectioning (Figure 6).

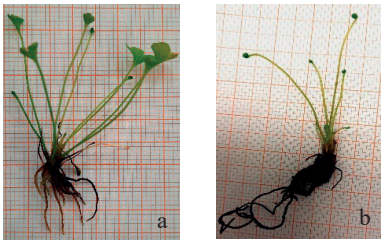


Figure 6. Undivided (a) and divided sporophyte (b) development

The growth rate reached the value of 6.25±0.47, close to that recorded for undivided sporophytes of 6.52±1.15 after one year of cultivation on MSFAV medium (Table 4). However, the number of leaves at the level of the divided sporophyte was not recovered, the difference being clearly superior in undivided sporophytes. However, at the root level, a higher root length was recorded in the divided sporophyte, but the difference was not significant.

Table 4. Comparison between gravimetrical and morphometrical parameters of divided and undivided sporophytes at twelve months of growth on MFAV medium

Variants	SGR	LN	LLL (cm)	SLL (cm)	LRL (cm)
Divided sporophytes	6.25±0.47	2.5±0.83	1.58±0.44	-0.28±1.13	2.98±1.14
Undivided sporophytes	6.52±1.15	6.33±2.5	2.28±0.92	-0.26±0.55	2.86±0.85

Obtaining gametophytes from leaves and roots fragments

After 20 days of inoculation the rootlets detached from sporophytes cultured on MFAV medium differentiated gametophytic prothalli, while leaf explants generated gametophytic-like structures after a few months (Figure 7).

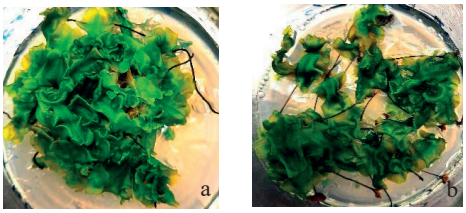


Figure 7. Aposporic gametophytes regenerated from rootlets (a) and leaflets (b)

Lawton (1936) reported the obtaining of diploid gametophytes (with antheridia and archegonia) and tetraploid sporophytes, in the species *O. regalis*, by inoculating, under sterile conditions, of explants from primary leaves of diploid sporophytes on Shieve minimal medium containing only 200 mg/l $Ca(NO_3)_2$. In contrast, the root tips did not differentiate into gametophytes.

In our experiment, the direct production of gametophytes from sporophyte tissue confirmed the occurrence of apospory. The induction of apospory demonstrates that the gametophyte can be generated from somatic tissues without the usual process of sporogenesis. The gametophyte formed by apospory retains the diploid number of chromosomes, unlike the usual gametophyte, which is haploid. In Nakamura and Maeda (1995) obtained aposporic gametophytes from fragments of excised sporophyte leaflets from the species *Lygodium japonicum* L., which were used for the isolation of protoplasts. Marimuthu et al., 2022, mention several factors that influence the induction and subsequent development of aposporic gametophytes including the detachment of organs from the mother plant, homogenization of sporophyte tissues, culture media with low or absent concentrations of sucrose.

Acclimation of sporophytes to potted soil

To ensure an efficient transition from the *in vitro* to the *ex vitro* environment, plants must progressively adapt to the low humidity, high light intensity, autotrophic nutrition and non-sterile conditions specific to greenhouses and experimental fields.

In order to obtain *O. regalis* plants, sporophytes obtained by *in vitro* culture were acclimatized to potted soil conditions. In a first experiment, three variants of both sterile and non-sterile substrate were tested. Subsequently, four more substrate variants were used to determine the adaptation of this species to different soil types. During the first month of acclimatization, a slight decrease in the weight of the sporophytes was observed, attributed to dehydration resulting from the transition to lower chamber humidity. Some leaves dried out, while the remaining ones expanded their foliar area. The acclimatization rate to *ex vitro* conditions, was 100%, after two months of potted culture, for all substrate variants except S1. This substrate proved to be unrecommended for this species, as all potted plants on this substrate did not survive (Table 5).

Table 5. The survival rate after two months in acclimatization conditions on different potted substrates

Substrate variants	% survival rate
Substrate for indoor plants S1	0
Sterile S1	0
Substrate for sowing and propagation S 2	100
Sterile S2	100
Substrate for ornamental shrubs S3	100
Sterile S3	100
Substrate for aquatic plants S4	100
Sterile S4	100
Substrate for acidophilous plants S5	100
Sterile S5	100
Substrate for cacti S6	100
Sterile S6	100
Natural peat substrate S7	100
Sterile S7	100

Moreover, other studies have also shown a 100% acclimatization of *O. regalis* plants obtained in vitro, to *ex vitro* culture conditions using sterile natural peat as a substrate with a pH of 6.5 (Makowski et al., 2016) or a 3:1 mixture of peat and perlite (Morini, 2000).

Potted plant growth

The development of potted plants under greenhouse conditions was monitored at 6 and 12 months of growth on two substrate variants: S2 containing 80% peat and 20% humus and S3 with bark enriched with nutrients and trace elements (Figure 8).



Figure 8. Potted plants at four and twelve months under greenhouse conditions

The best growth rate in terms of weight gain was observed on S2, both at 6 and 12 months (Table 6).

The variants with the autoclaved substrates recorded lower values than those in which the non-sterile substrate was used.

The survival rate of potted plants after 12 months of cultivation decreased, reaching from 83% for plants grown on substrates of variant 2 to 50% for plants grown on sterile substrate of variant 3.

Table 6. The growth and survival rate of potted plants on different substrates at 6 and 12 months in greenhouse

Substrate	RL (cm)		RV (µl)	
	6 m	12 m	6 m	12 m
S2	3.55±1.1	23.42±1.2	358.33±20.4	1260±418.3
Sterile S2	2.76±1.3	22.68±1.5	416.66±40.8	1520±670.8
S3	1.53±0.5	19.52±1.1	325±41.8	1375±150
Sterile S3	2.22±1.3	19.33±0.5	330±27.3	1366.66±57.7

The number of leaves was reduced from 8-11 leaves/sporophyte to 4-5 leaves/potted plant after one year of greenhouse culture.

The leaves undergone shape and size changes, starting to resemble the fronds from natural conditions (Figure 8).

The dimensions of the largest and smallest leaves also showed better growth on S2 substrate (Table 7).

Table 7. Morphometrical leaf parameters of potted plants on different substrates at 6 and 12 months in greenhouse

Substrate	LN		LLL (cm)		SLL (cm)	
	6 m	12 m	6 m	12 m	6 m	12 m
S2	-4±2.8	-5.2±2.7	2.86±0.4	5.76±1.4	2.9±0.5	1.2±0.8
Sterile S2	-1±1.4	-3±2.5	1.66±0.5	4.94±0.5	1.63±0.7	0.74±0.7
S3	-4±2.9	-4.5±2.8	1.12±1.7	3.72±1.8	2.55±0.9	0.04±1.1
Sterile S3	-2.8±2.5	-5.33±3.7	1.6±0.6	5.06±0.5	1.52±1.7	2.13±1.2

Compared to the vegetative system, notable development occurred at the root level between 6 and 12 months (Figure 9).



Figure 9. Development of roots system at 12 months after transfer to potted soil

The radicular volume was 4 times greater than at 6 months, while the root length reaches from 3.55±1.15 cm at 6 months to 23.48±1.28 cm at one year for substrate variant 2, which was more advantageous than the other variants (Table 8). For variant 2, the radicular system volume was higher for the sterile substrate, than the non-sterile one. For variant 3 this sterile-unsterile difference was not preserved.

Table 8. Morphometrical root parameters of potted plants on different substrates at 6 and 12 months in greenhouse

Substrate	SGR		SP (%)	
	6 m	12 m	6 m	12 m
S2	3.77±0.94	2.11±0.42	100	83
Sterile S2	2.19±1.28	1.93±0.27	100	83
S3	3.20±1.29	1.93 ±0.18	100	66.67
Sterile S3	2.61±0.78	1.99±0.13	83.33	50

CONCLUSIONS

In conclusion, the results of our study indicate that the species *O. regalis* prefers nutritive media with low nutrient concentrations, a characteristic common to the majority of fern species, having though a specific requirement for nitrogen-poor environments for both gametophyte development and sporophyte differentiation and development. The high percentage of acclimatized plants suggests that the species *O. regalis* can also adapt to other types of substrate depending on environmental conditions, offering opportunities for the restoration and conservation of ecosystems by reintroducing the species into natural environments.

ACKNOWLEDGEMENTS

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ENVIRONMENTAL FACTORS AFFECTING THE CONDITION OF *PLATANUS* x *ACERIFOLIA* (AITON) WILLD. URBAN STREET TREES IN SOFIA

Svetlana ANISIMOVA[1], Elena TSVETKOVA[1], Gergana ZAEMDZHIKOVA[2]

[1]University of Forestry, 10 St. Kliment Ohridski Blvd, 1797, Sofia, Bulgaria
[2]Forest Research Institute-BAS, 132 St. Kliment Ohridski Blvd, 1756, Sofia, Bulgaria

Corresponding author email: sanisimova@ltu.bg

Abstract

Street trees are the most vulnerable element of the urban green infrastructure and are subjected to many stressors. In recent years, there has been a noticeable increase in Platanus x *acerifolia (Aiton) Willd. presence in the streetscapes of Sofia. The aim of this research is to identify important environmental abiotic and anthropogenic factors as well as biotic ones affecting its condition. The following influencing factors of tree performance in highly urbanized streetscapes were analyzed: meteorological indicators, air pollution, streetscape morphology, maintenance practices; and pests and diseases. Data for 1,824 street trees were collected by a field survey in alignments of the primary and secondary street network, distributed in commercial zones and residential areas, in the whole variety of possible orientations of streets. The analysis indicated the dominant presence (86.68%) of young trees (DBH < 25 cm), while 4.22% were over 50 cm in diameter. According to our research, the key factors that contributed to the poor performance, structural damage, and health decline of London plane street trees were drought and poor arboricultural practices. A considerable proportion of mature specimens (74.09%) had unclosed pruning wounds with diameter over 25 cm. Late removal of large branches of mature trees for crown lifting, reduction, or removal of structural defects led to decayed pruning wounds (53.64%) and development of water sprouts (20%), respectively. The lower trunk cavities in 13.18%, leaning trunk in 8.18%, branch dieback in 52.27% and unbalanced crowns in 11.36% of investigated mature specimens make them potentially hazardous. Some of young specimens displayed different human-caused damage (7.27%). Regarding the biotic factors, the degree of damage caused by pests and diseases was low – an average of 15%. The results revealed a statistically significant difference in DBH between two groups of young trees – one in median grass strips with a irrigation system and the other without. The analysis of heavy metals in the leaves as an indicator for pollution showed a significant correlation between the accumulation of Al and Pb, Ni and Cr (r=0.91÷0.96) and between Pb and Cr (r=0.98). The results provide guidance for the management of Platanus* x *acerifolia street trees in the urban green infrastructure in order to enhance their environmental benefits.*

Key words: *London plane; green infrastructure; stressors.*

INTRODUCTION

Platanus x *acerifolia* (Aiton) Willd. is among the most commonly planted species in urban areas, favoured for its hardiness and ability to thrive in adverse urban conditions (Caneva et al., 2020**;** Sanusi & Livesley, 2020; Galle et al., 2021; Roman & Eisenman, 2022; Wang et al., 2023). London plane trees demonstrate high environmental stress tolerance in streetscapes in terms of resilience to water stress and toxic ambient urban pollutants (Tiwary et al., 2016; Haase & Hellwig, 2022). The species is valued and widely used as a street tree in urban environments due to its numerous ecological benefits. Platanus trees provide significant ecosystem services, including air purification, stormwater runoff reduction, urban heat island mitigation, microclimate regulation, and thermal comfort improvement, contributing to the enhancement of biodiversity by providing habitat for urban wildlife (Wang et al., 2018; Wood & Esaian, 2020; Duval et al., 2022; Shen et al., 2023).

The economic, aesthetic, and social benefits of London plane street trees are substantial, with significant contributions to energy savings, increased property values, enhanced aesthetic appeal of urban areas, and improved quality of life (Rotherham, 2010; Wang et al., 2018; Xiong et al., 2019).

Platanus x *acerifolia* plays a significant role in the urban green spaces of the city of Sofia. It is one of the most prevalent species in the whole city street network, especially among the plantings in the last decades (Anisimova, 2023).

However, the health of plane trees is compromised by climate change effects (Dobrescu & Fabian, 2017; Sanusi & Livesley, 2020), pests and diseases (Pelleteret et al., 2017), which severely impact their vitality and reduce their ecosystem service benefits when they are most needed. Urban environments pose several challenges for Platanus trees, including soil compaction, pollution, and limited water availability. These factors can lead to health issues and reduced longevity (Gillner et al., 2015; Ordóñez et al., 2018).

The aim of this research is to identify the most important environmental abiotic, biotic, and anthropogenic factors affecting the overall performance of London plane urban street trees in the city of Sofia.

MATERIALS AND METHODS

The field-based survey was carried out during the period from 2021 to 2024, which included street alignments of all classes of the primary and secondary street network of the city of Sofia – II (Urban highways with intermittent traffic regime); III (Regional arterial streets); IV (Main streets), V (Collectors).

Overall performance of trees

The attributes that have been examined, collected and evaluated by field studies to characterize the overall performance of trees were: diameter at breast height (DBH); stem injuries and problems, crown damage and defects, and leaf damage by pests and diseases. Along with the tree phytosanitary status assessment, the poor maintenance practices and conflicts with the city's infrastructure (width of the sidewalk, distance of the stem from the street curb and the buildings) were considered.

The condition of the foliage of each specimen was visually assessed according to the degree of defoliation and degree of foliage transparency (Eichhorn et al., 2020) on a scale from 0 to 100%, with a step of 5%.

The following obvious tree defects were examined: leaning trunk, wounds and cracks on the bark and stem, wood decay in the lower trunks and cavities, fruiting bodies of a basidiomycete (basidiocarp), and bacterial bleeding canker. Crown states were analyzed for an unbalanced crown, wood decay in unsealed pruning cuts, broken branches, dieback of twigs or branches, and epicormics (water) sprouts.

Meteorological and air pollution data

The abiotic factors that have been analyzed were temperature, relative humidity, and air pollution. Data on hourly values of meteorological variables: air temperatures (°C), relative humidity (%), and average hourly concentrations of PM_{10}, $PM_{2,5}$ and O3 for the period 2015-2024 from 5 Automatic Measuring Stations of the Executive Environmental Agency (EEA) were used.

Samples for leaf analysis were taken from 15 trees from 5 streets from II and III class. The sampling was carried out in August.

Statistical analysis

Descriptive statistics and single regression analysis were performed. Correlation coefficients (R) at selected significance thresholds of 95% and 99% probability were determined. For significant relationships, linear models of the type: y=b.x+a were inferred. Excel was used for mathematical-statistical and graphical data processing.

RESULTS AND DISCUSSIONS

Street tree data for 1,824 tree specimens of *Pl.* x *acerifolia* were collected by a field survey in street alignments of all classes of the primary and secondary street network of the city of Sofia – II (17.84%), III (62.79%), IV (6.75%), V (12.62%).

The street plantings were located in different administrative territorial units of Sofia Municipality. The dendrometric indicator DBH of the surveyed street trees serves as an approximate assessment of the age of the specimens and was classified into categories (Figure 1). The analysis of DBH indicated the dominant presence (86.68%) of young trees (DBH < 25 cm), while 4.22% were over 50 cm in diameter. Most of the young trees were planted in the last two decades.

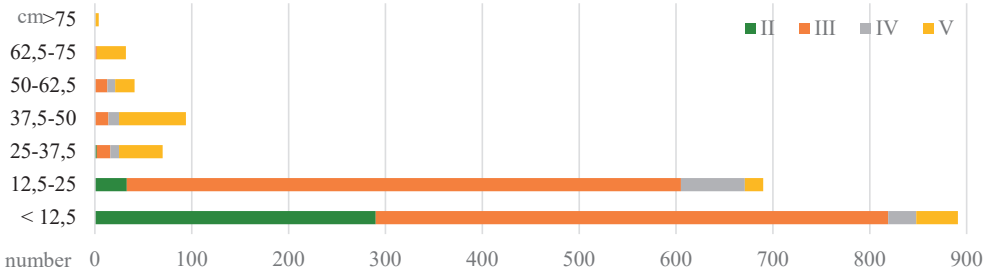


Figure 1. DBH class structure of *Platanus* x *acerifolia* trees for the main street classes

The age of the studied specimens ranged between 10 and 100 years.

The following influencing factors of tree performance in highly urbanized streetscapes were determined: abiotic, incl. soil conditions, meteorological indicators, air pollution, streetscape morphology, and maintenance practices; and biotic, including pests and diseases.

Abiotic and anthropogenic factors

Streetscape morphology and growing medium

The tree alignments were distributed along 20 streets and 10 boulevards in commercial zones and residential areas (Figure 2).

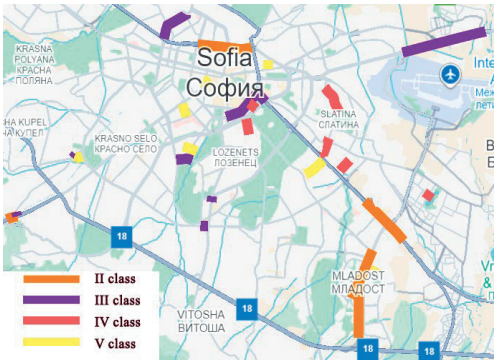


Figure 2. Surveyed streets in Sofia

The trees were located in the whole variety of possible orientations of streets – E-W (44.04%), N-S (22.32%), NW-SE (13.13%), NE-SW (20.51%) – and physical characteristics of the built environment. The width of the sidewalks varied between 2 and 8 m. 57.84% of the specimens were planted in median strips or grass verges (green strips between the road and sidewalk), while the rest were planted in single tree pits in concrete paving on streets with different building densities. There were limitations to this study related to the inability to analyze soil and light factors in dense urban environments. In relation to the insolation regime, some of the specimens on the same sidewalk of the streetscape were flanked by multi-story buildings creating a canyon effect, while others were exposed to total irradiation.

Soil conditions and the tree-planting sidewalk cut-out area also varied across different sites. The trees were planted directly in the ground without any soil improvements to the growing medium – no amendments or application of structural soil. The application of de-icing salts on roads and pavements in winter is also part of municipality maintenance practices. However, these attributes vary from year to year, seasonally, and depend on the street zone. About 1/4 of the trees in the median strips were provided with irrigation systems.

Data analysis of a case study involving two groups of 15-17-year-old fully irradiated London plane trees grown along the same boulevard in Sofia – one group in median strips/grass verges with a surface irrigation system and the other without – revealed a statistically significant difference in DBH between the two groups ($p = 2.73E-11$) and crown volume, respectively. A greater range of DBH was observed in the non-irrigated area (Figure 3): more than 1/2 of the examined specimens had a DBH between 8 and 10 cm, while only 1/4 of the trees reached a diameter of 15-16 cm. On the other hand, 3/4 of the trees under irrigated conditions exceeded these dimensions. Some individual specimens in the non-irrigated grass strips reached sizes around and slightly above 22 cm. It was found that these trees were adjacent to a drainage channel. The average diameter of the trees under irrigated

conditions was about 5 cm larger. The mortality rate among non-irrigated specimens ranged between 10-15%. Optimized irrigation management, particularly with respect to tree phenology, could contribute to maximizing plane tree performance and survival in urban areas under climate change (David et al., 2018; Claude et al., 2024).

Plane trees planted in constrained environments, such as small tree pits or areas with concrete paving, tend to exhibit poorer health (Tan et al., 2022). Site factors variably affected stem caliper of plane street trees, which increased with a greater sidewalk cut-out area (Sherman et al., 2016). Soil conditions that influence tree performance in highly urbanized streetscapes frequently include: lack of soil volume available for adequate root growth; low soil nutrient and organic matter content; soil compaction, which hinders root development and water availability; elevated soil salinity, which causes osmotic stress to trees and frequently manifests as leaf chlorosis; high soil alkalinity, which can influence nutrient availability; and either poor drainage or low soil water-holding capacity.

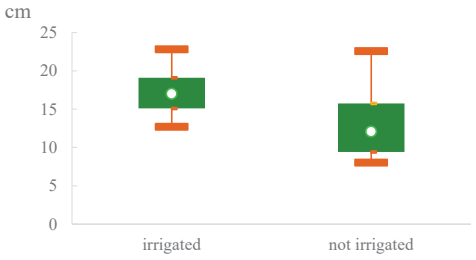


Figure 3. Box-plot diagrams of DBH of irrigated vs non-irrigated specimens

High soil salinity and alkalinity, along with physical damage and excessive sunlight exposure, have been linked to the decline of *Platanus* trees in urban settings (Ordóñez et al., 2018).

The importance of edaphic and irradiation factors has been recognized and deserves future investigation.

In a study of "hot spots" in terms of physiological stress and traffic pollution in Bucharest, *Pl.* x *acerifolia* was found to be one of the species less affected by adverse microclimate and edaphic urban conditions. The results indicated that younger specimens suffered more under heavy traffic conditions (Dumitrașcu et al., 2010). Dendrochronological analyses revealed that the species was found to be less affected by a high degree of urbanization and exhibited higher drought tolerance, making it suitable for urban planting in temperate climates (Franceschi et al., 2023).

Human-induced damage

Pruning represents one of the most important maintenance practices for street trees. Its main objectives are improving safety, tree stability and health status, ensuring traffic clearance, and resolving conflicts with infrastructure. Some of young specimens displayed different human-caused damage, such as physical injuries to the crown, stem or root collar from vehicles or pedestrians, improper staking and arboricultural practices, and injury to the root collar area by grass trimmers (7.27%) (Figures 4 and 5).

The results show that a considerable proportion of mature London plane specimens (74.09%) had unclosed pruning wounds with diameter over 25 cm as a consequence of some improper pruning methods and techniques. Late removal of large branches of mature trees for crown lifting, reduction, or removal of structural defects led to decayed pruning wounds (53.64%) and development of water sprouts (20%), respectively (Figures 4 and 5). The process of callus and woundwood production (compartmentalization) is long and sometimes unsuccessful. Additionally, the lower trunk cavities in 13.18%, leaning trunk in 8.18%, branch dieback in 52.27% and unbalanced crowns in 11.36% of investigated matue specimens make them potentially hazardous.

The implementation of good formative pruning of recently planted *Pl.* x *acerifolia* street trees was found to be cost-effective, with lower costs compared to structural pruning of mature trees.

Improper pruning often leads to structural defects and health issues which can compromise the structural integrity of trees, making them more susceptible to failure. Pruning should be consistent with the biology of the species and good arboricultural practices. *Pl.* x *acerifolia* street trees, with their rapid growth, require a lot of pruning work to establish strong structural framework and maintain it to maturity (Ryder & Moore, 2013).

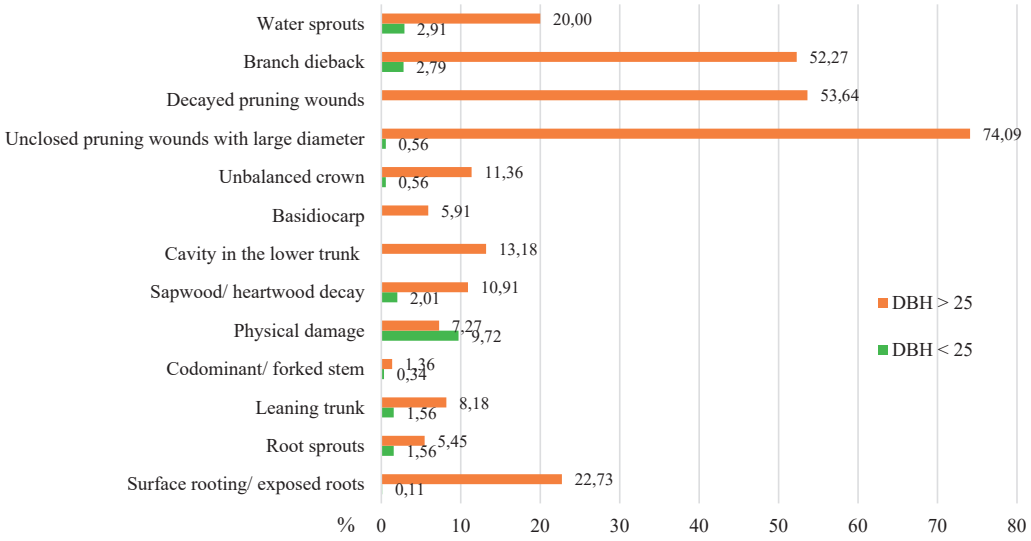


Figure 4. Percentage *Platanus* x *acerifolia* trees with structural defects, damage, and health issues

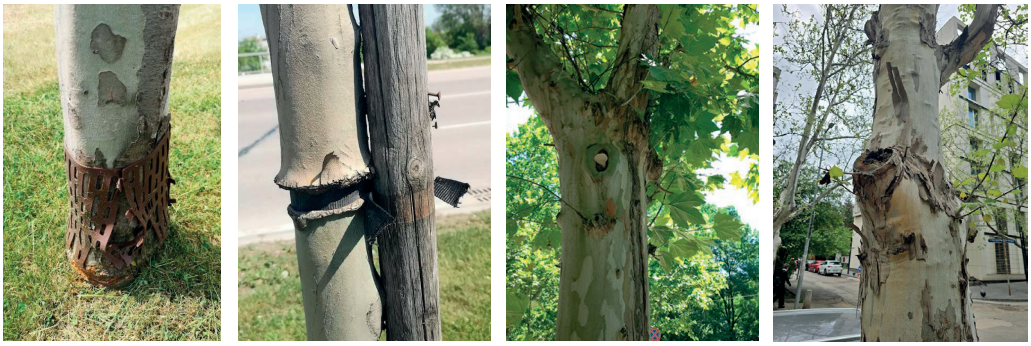


Figure 5. *Human-induced damage* of *Platanus* x *acerifolia* trees

Regular, moderate pruning of the London plane is recommended to maintain tree health and structure. This approach helps optimize the tree's ecosystem services, such as increased crown carbon sequestration and air quality improvement (PM_{10} capture). Pruning represents an opportunity to increase the crown carbon stock by up to 65%, taking into account the full reestablishment of the crown. Over-pruning can lead to increased susceptibility to diseases, branch dieback occurrence, and reduced capacity to provide ecosystem services (Muscas et al., 2024). Disease spread in *Platanus* street trees can be prevented through appropriate management and planting practices. Proper pruning, larger pits with less concrete paving, and the presence of tree grates or guards can impact the health condition of trees (Tan & Shibata, 2022). The results of this study are consistent with recent findings about the adverse effects of improper pruning practices on the health status and aesthetic value of horse chestnut street trees in Sofia (Pencheva & Anisimova, 2016).

Meteorological indicators

In the present study, the analysis of daily maximum temperature data for Sofia over a 10-year period (2015-2024) showed extremes of up to 48.1°C. These heat waves were recorded mainly from the last ten days of July to the second ten days of August. During these periods of elevated summer temperatures, leaf colour modification and early leaf drop of the plane trees were observed, especially in the areas with no irrigation. Extreme heat, such as heatwaves,

can lead to canopy leaf loss in *Platanus* x *acerifolia*, reducing ecosystem service benefits, such as shade cooling and human well-being in urban areas. A study in Melbourne observed 30-50% canopy loss in London plane trees following a heat wave with temperatures above 43°C (Sanusi & Livesley, 2020).

During the winter period, daily minimum temperatures in the range of -10 to -20°C were found. These low temperatures, combined with inversions that trap air pollutants in the ground layers, adversely affect the health of plane trees. Fluctuations in temperature conditions in urban environments were common during the study period. Temperature amplitudes in excess of 20°C per day were observed. This was especially true for the end of February, the second half of March and April, as well as during the first ten days of August.

The minimum value of relative humidity in the annual cycle was observed from June to September. During the same period, the onset of the dry period was expected. There were quite a few cases of relative humidity below 50%, and in certain periods, this value dropped below 30%, i.e. an indicator for atmospheric drought. In general, drought conditions exacerbate the vulnerability, especially of young plane trees, because, along with earlier foliage loss, they suffer from leaf chlorosis during drought periods and heat waves (Akhbarfar et al., 2023). Drought stress leads to a decline in chlorophyll content, net assimilation rate, and relative water content, which are particularly critical for the health and growth of young trees. Severe drought has been found to induce defoliation in London plane trees when extractable soil water content decreases below 25% (Claude et al., 2024). During periods of heat waves and drought, the maintenance of green alignments in Sofia rarely includes watering. The UHI effect, prolonged heat waves in combination with drought, lead to heat stress on street trees. Plane trees respond to water deficit with a time lag, resulting in lower growth in the years following the drought (Hirsch et al., 2023). The consequences of these urban conditions include failure to establish, retarded growth rates, tree decline and mortality.

The health status of the urban population of *Pl.* x *acerifolia* has been negatively and most significantly influenced by long-term precipitation amount, higher precipitation amount in May, and a higher number of freezing days in winter. During the period of the highest damage, the specimens were additionally affected by the fungal pathogen *Apiognomonia veneta* (Sacc. et Spegg.) Höhn (Gregorová et al., 2010). Its spread is heavily influenced by high humidity and precipitation (Ivanová et al., 2010).

Air pollution

In Bulgaria, particulate matter (PM_{10} and $PM_{2,5}$) has been identified as a major pollutant adversely affecting the near-surface atmosphere and air quality. The monitoring of air pollution in Sofia for the last decade during the vegetation period reported peak hourly concentrations per day of PM_{10} that exceed 100 μg/m³. PM pollution is most often due to dust deposited on the streets as well as to deteriorated green areas in the capital, traffic and construction. Domestic heating with solid fuels during the winter months is also a source of dust pollution. Mineral dust transport from long distances, mostly originating from the Sahara Desert, is also a significant contributor. More than 104 days of the vegetation period have been reported with atmospheric circulations carrying Sahara Desert dust (AHB, 2022, 2023). From March to the first half of April, PM_{10} values exceeded 250 μg/m³. Towards the end of June, levels in the range of 450-550 μg/m³ were recorded in 2016 (543 μg/m³) and 2019 (464 μg/m³), respectively. In the second half of August, pollution was also detected reaching maximum hourly levels of 513 μg/m³. The dynamics of maximum hourly mean concentrations of $PM_{2,5}$ largely duplicate the PM_{10} load at the beginning of the plane vegetation period in March. At the beginning of the study period, peak values in March reached 134 μg/m³, and in June – 96 μg/m³.

These fine particles deposited on the leaf blade negatively affect physiological and metabolic processes of plane trees. High concentrations have also been recorded at the end of the vegetation period and in fall. Values for the autumn season reach levels of up to 87 μg/m³.

Street trees have been found to improve thermal comfort by lowering air temperatures and reducing PM levels, thereby improving outdoor urban environmental conditions (Miao et al.,

2023). However, the presence of street trees can also prevent the dispersion of air pollutants in street canyons, leading to the accumulation of street pollutants (Wang et al., 2024).

One of the main sources of heavy metal emissions in Sofia during the vegetation season is traffic. According to the latest data, more than half of the PM pollution is due to vehicles in the capital. Airborne PM in Sofia is loaded with polymetallic substances. Plane leaves coated with tiny, fine, stiff hairs retain significant amounts of fine PM, and this is also true for heavy metals. This is how they contribute significantly to improving air quality. The study found that 33% more dust was accumulated on the leaves of street trees in comparison to those of park specimens: 1.05 mg/cm^2 in heavy traffic vs. 0.79 mg/cm^2 in a park area, respectively.

Information on air pollution in hotspots of the city can be easily extracted from the concentration of heavy metals in the assimilation organs of trees. The analysis of the results showed a significant positive linear correlation between the accumulation of Al in leaves, on the one hand, and that of Pb, Ni and Cr on the other. Moreover, a strong positive linear correlation was found between the accumulation of Pb and Cr in the leaves of street plane trees (Table 1).

Table 1. Correlation coefficients and coefficients of determination and linear equations of significant relationships between heavy metal content in *Platanus acerifolia* leaves

	Al	Zn	Cu	Ni	Pb	Cd	Cr
Mn	0,75	-0,03	-0,29	0,74	0,80	0,74	0,77
Al		-0,50	-0,39	**0,94***	**0,96****	0,43	**0,91***
Zn			0,21	-0,37	-0,29	-0,01	-0,26
Cu				-0,66	-0,21	-0,74	-0,02
Ni					0,87	0,61	0,76
Pb						0,34	**0,98****
Cd							0,22

*Legend: *p < 0,05; **p < 0,01*

R^2 = 0,8802
Ni = 0,0196.Al - 0,642
Al = 44,796.Ni - 39,604
R^2 = 0,9177
Pb = 0,0069.Al - 0,2742
Al = 132,9.Pb + 43,888
R^2 = 0,8191
Cr = 0,0187.Al - 0,5573
Al = 43,772.Cr + 40,768
R^2 = 0,9617
Cr = 2,813.Pb + 0,1496
Pb = 0,3419.Cr - 0,0377

Tropospheric ozone can cause some damage to tree leaves and reduce biomass production, influencing tree decline, respectively (Xu et al., 2015). The calculated values of AOT40 for the study period, averaged over a 5-year period, exceeded the long-term target standard of 6,000 µg/m^3.h by 1.08 to 1.33. Between 2017-2021, 6 ozone days were recorded with mean hourly pollutants above 180 µg/m^3.h.

Biotic Stress Factors. Damage caused to Pl. acerifolia by biotic factors

Platanus acerifolia is susceptible to various pests and diseases, which – along with factors, such as pollution and climate change – contribute to the likelihood of death and failure of plane trees (Pelleteret et al., 2017).

Three insect species and two fungal pathogens causing damage to the crown of plane street trees were established, respectively: *Corythucha ciliata* (Say, 1832) (Hemiptera: Tingidae), *Phyllonorycter platani* (Staudinger, 1870) (Lepidoptera: Gracillariidae), *Metcalfa pruinosa* (Say, 1830) (Hemiptera: Flatidae), *Apiognomonia veneta* (Sacc. et Speg.) Höhnel. and *Erysiphe platani* (Howe) Braun & Takam. The species composition of pests and fungi, as well as the degree of damage caused by them, are given in Table 2. The degree of damage caused by pests and diseases in the study area and period was low – an average of 15%. Regarding the biotic factor, it can be concluded that the health status of the plane tree is good, because all inventoried trees had an average degree of damage ≤ 20%.

Table 2. Damage caused to *Platanus acerifolia* street trees by biotic factors

Species	Assessed trees, N	Average degree of damage, %
*Corythucha ciliata**	190	16
*Phyllonorycter platani**	51	16
*Metcalfa pruinosa**	74	13
*Apiognomonia veneta***	85	17
*Erysiphe platani***	17	8

*insect pests **fungal pathogens

The relative share of plane trees affected by the insect pests is as follows: *C. ciliata* (45.6%) – almost half of the inventoried specimens, while *Ph. platani* (12.2%) and *M. pruinosa* (17.7%) were represented with a low frequency among street trees. *Corythucha ciliata* is an invasive exotic pest native to North America that is widely distributed in Europe, as well as in Bulgaria (Dobreva et al., 2013). It is known that the leaf damage caused by nymphs and adults of *C. ciliata* and larvae of *Ph. platani* can cause significant leaf loss in plane trees, as well as physiological and aesthetic damage (Mutun, 2009; Tóth & Lakatos, 2018; Florian et al., 2022). Here, the degree of damage caused by these pests to London plane street trees in Sofia was negligible (Table 2). The development and fecundity of *C. ciliata* were found to decrease significantly as temperature increased. The optimal developmental temperature was determined to be 30°C, indicating a potential increase in pest infestation under extreme heat conditions (Ju et al., 2011; Lesovoy et al., 2023). For the first time in Bulgaria, we observed nymphs and adults of M. pruinosa on young shoots of plane trees. M. pruinosa is a very polyphagous native insect in North America and is currently a serious pest in Europe (Strauss, 2010; Chireceanu & Gutuie, 2011).

The pathogen *A. veneta* – a causal agent of plane tree anthracnose, was found to infect 20.4% of the inventoried trees, while powdery mildew (*E. platani*) infected only 4.1% of the trees. The degree of damage caused by these fungal pathogens was 17% and 8%, respectively (Table 2). *Apiognomonia veneta* is one of the most frequent and severe diseases affecting plane trees in Europe, leading to a weakening of the trees (Tello et al., 2005; Ivanová et al., 2010; Tubby & Pérez-Sierra, 2015). A negative effect on the health status of urban plane tree by the fungal pathogens *A. veneta* and invasive pest *C. ciliata* in Sofia was reported by Georgieva et al. (2023).

Trees in warmer urban areas are more water-stressed and in worse condition, which can exacerbate pest infestations. The stress from heat island effects and increased impervious areas surrounding them makes trees more susceptible to pests, leading to a vicious cycle of declining tree health and increasing pest problems (Dale et al., 2016).

Effective management, including regular maintenance and monitoring, is crucial to mitigate the risks of tree failure and ensure the health of *Platanus* street trees (Wang et al., 2023).

CONCLUSIONS

Platanus street trees play a vital role by providing numerous benefits to urban environments. However, their successful integration and long-term contribution require strategic planning and implementing effective management practices to maximize their positive impact in the urban green infrastructure. According to our research, the factors that contributed the most to the poor performance, structural damage, and health decline of plane street trees were drought and poor arboricultural practices. Therefore, best practices for sustainable management of London plane street trees should include: provision of enough space in the street design for development; formative pruning to establish strong structural framework, that should be maintained to maturity; irrigation of young specimens; systematic monitoring, and early detection of pests and diseases.

Further investigation into other factors that have been suggested as possible causes for poor street tree performance and their cumulative effect on urban London plane decline should be conducted.

The results from the research could be used to guide decision-making on species selection and management strategies.

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BUTTERFLIES IN THE URBAN LANDSCAPE - CASE STUDY: TEILOR PARK FROM BUCHAREST, ROMANIA

Monica Luminița BADEA[1], Eugenia PETRESCU[2], Alexandru ȘTEFAN-FOTIN[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Văcărești Natural Park Administration, 3 Uioara Avenue, District 4, Bucharest, Romania
[3]S.O.S. URGENTVET SRL, 19 Dimitrie Cantemir Blvd, District 4, Bucharest, Romania

Corresponding author email: petrescu_eugenia@yahoo.com

Abstract

In the Teilor Park (Linden Tree Park) in Bucharest, we carried out observations on the species of butterflies between July 2022 and November 2024. Butterflies are appreciated for their beauty, being a source of inspiration, they are pollinators and indicators of environmental quality. Pesticide pollution and urbanization through fragmentation of habitats cause a decrease in the diversity of insect species, including butterflies. We made a list of plant species in the park, to identify possible food sources for larvae and adults. Periodic visits carried out along the transects organized along the alleys, as well as observations on the slopes, noting: the species of butterflies, the number for each species and the plants on which the feeding behaviour of the adults was observed. We took photos of the butterflies and especially photos of the butterflies visiting flowers. We were able to identify 21 species of butterflies. As the investigated area is relatively small (7 ha), we can appreciate that the park supports relatively well the diversity of butterfly species.

Key words*: butterflies, biodiversity, urban landscape.*

INTRODUCTION

We are currently witnessing a drastic decline in biodiversity. The European Union intends to restore biodiversity and reduce the causes of biodiversity loss by 2030 (https://eur-lex.europa.eu/RO/legal-content/summary/eu-biodiversity-strategy-for-2030.html).

The biomass of insects, including pollinators, has decreased by more than 70%, and the number of species has decreased between 20 and 50% in Europe and North America in the last 30 years, as a result of anthropogenic activities, such as the use of pesticides, the destruction and fragmentation of habitats, but also due to climate changes (Rákosy and Goia, 2021). In Romania, in the last 30 years, the number of insects, including butterflies, has decreased, and one of the factors causing it is urbanization (Székely, 2024).

Although many works illustrate the negative effects of urbanization on insect populations and on the composition of the insect community, there are also studies indicating that cities can also shelter and even favor a considerable number of native species (Theodorou, 2022). Lepidoptera totals 180,000 species, of which butterflies constitute only 10%, the remaining 90% being moths (Rákosy, 2024).

Among Lepidoptera 141,600 species are known as pollinators, feeding on flower nectar, namely 18,500 species of butterflies (Rhopalocera) and 123,100 species of moths (Heterocera) (Ollerton, 2017). Butterflies are good indicators of environmental quality (Rákosy, 2024), very sensitive to urbanization (Han et al., 2021).

In Romania, of the 4,000 known species of Lepidoptera, 202 are butterflies (Rákosy, 2024).

Recent specialized literature, according to Kowarik et al., 2020, highlights the importance of cities to biodiversity and even to endangered species, despite the pressures that the urban environment creates on wildlife; for a healthy life for its inhabitants, both physically and psychologically, it is essential that the development of cities is sustainable, with a care for biodiversity and awareness of the fact that we share these urban habitats with animals as well. Parks could provide suitable habitats for fauna species, if we take into account the species that are planted, but also that the wild plants, many of which are considered weeds

that must be removed from the landscape, would play their part in drawing pollinating insects in particular.
The article presents the results of a study on butterflies in a park from Bucharest, in order to attempt an assessment of the potential of an urban park in maintaining the biodiversity of butterfly species.

MATERIALS AND METHODS

Teilor Park is one of the few parks recently created in București, sector 3. The land on which the current park is located was previously a wasteland situated at the waste-filled junction of Brățării Street and Gura Siriului Road. The park opened to the public in May 2020. With an area of approximately 7 ha, the park is located on a small artificial hill, at the junction of Brățării Street and Drumul Gura Siriului (https://primarie3.ro/index.php/spatii_verzi, accessed 20.03.2024, Figure 3). The Park is equipped with sports fields, playgrounds for children, an artificial lake, two floating islands covered in reeds. The lakeshore is bordered by weeping willows and metasequoia.
Măriuca Cristiana Hogea, the engineer hired by the City Council of District 3 between 2022-2024, told us that, during this period of time, 900 lime trees and 4,000 lavender bushes were planted on the hill in Teilor Park. Shrubs such as *Juniperus* spp. were also planted, as well as 12,000 bushes of *Hibiscus syriacus* to create hedges. (Figures 1, 2)
With the Google Maps mobile application, we took the approximate geographical coordinates at 4 corners, and the park forms a rectangle, with the sides arranged parallel to the streets Drumul Gura Siriului (eastern side), Brățării (northern side), Vărăști (the western side), Poștașului (southern side). The coordinates obtained in the field are: 44.417905 N; 26.189149 E at the entrance from Drumul Gura Siriului Street, the southern side; 44.420198 N; 26.190 453 E for the entrance from the junction between Drumul Gura Siriului and Brățării Street; 44.420959 N; 26.187991 E at the entrance from Brățării adjacent to Vărăști Street and 44.418548 N; 26.186 915 E in the green corner of the side parallel to Vărăști street.
Observations were carried out between July 2022 and November 2024, with visits made at least once a month. Transects were established along the paths, totaling approximately 800 m (Figure 3), but simple observations were also made.



Figure 1. Planting of lavender in Teilor Park, photo by Cristiana Măriuca Hogea



Figure 2. Lavender and linden tree in Teilor Park

The species of plants and butterflies observed were noted and photos were taken using a Nikon D 3300 camera with a Sigma 105 mm 1:2:8 DG Macro HSM lens; all butterfly photos in this paper belong to the authors. Field guides were used to identify the species, such as the work of Sîrbu et al., *Flora ilustrată a României* of 2013 and the book of Rákosy, *Fluturii din România. Cunoaștere, protecție, conservare*, from 2013, and we used, for the most part, the names of the species and the taxonomic classification used in these books. The species encountered and the number of individuals of each species were recorded on the transects. For easier data collection, in 2024 we also used

the *Spobs* mobile application, created and developed by Andrei Conțiu, which allowed us to record adult insects in association with the plants where we saw them feeding on nectar.

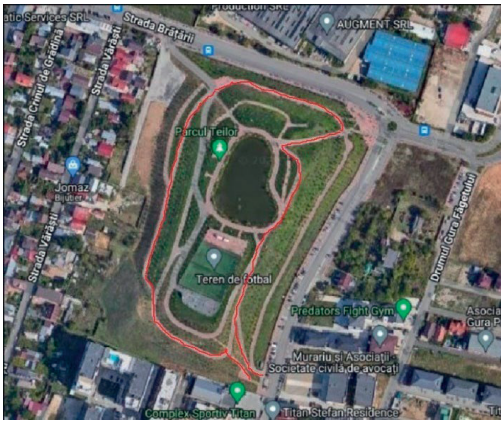


Figure 3. The Teilor Park- monthly transects in 2024 (with red)

RESULTS AND DISCUSSIONS

Between 2022-2024 we identified 21 species of butterflies in Teilor Park, belonging to the families Hesperiidae, Papilionidae, Pieridae, Lycaenidae and Nymphalidae. In 2022, 13 species of butterflies were observed, in 2023 only 10 species. In 2024 we found the species observed in previous years, to which 7 more species were added, 21 species in total (Table 2, Table 4). The best represented was the Lycaenidae family (29%), totaling 6 species, followed by Pieridae (24%) and Nymphalidae (24%), each with 5 species (Figure 4, Table 1, Table 2, Table 4).

Comparatively, in another park in sector 3 (Petrașcu Park), with an area of 3 ha, Albu and Albu (2016) identified 13 species of butterflies belonging to 5 families, over a period of 12 years (1970-1982). Our observations over a period of 13 years (2011-2024) in Petrașcu Park totaled only 7 species (Ștefan-Fotin and Petrescu, unpublished data). Currently, Petrașcu Park is a small park with mature trees, providing shade; under the trees and in open areas the lawn is maintained by mowing. Teilor Park, a new park, with young trees that do not maintain shade, presents sunny slopes, on which lavender bushes are planted, among which, between hedges, spontaneous plant bloom, but also lawns with flowers that attract pollinators, such as white and red clover.

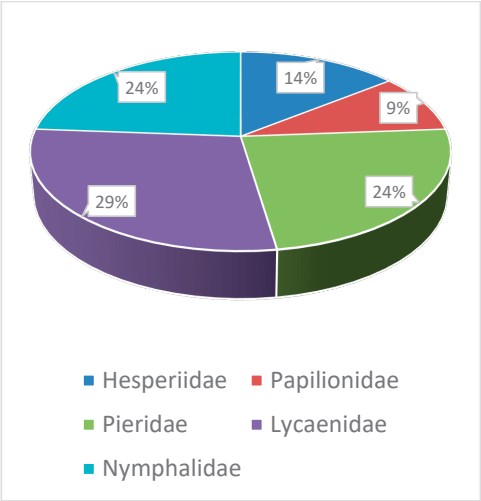


Figure 4. Percentage representation of butterfly families in Teilor Park

The study for the Văcărești Natural Park, the first urban protected area in Bucharest and the largest green space in the city, conducted by Popescu (2019) between April and October 2018, included a list of 27 butterfly species for a much larger area, of 183 ha, but our observations from recent years indicate many more species in Văcărești (Ștefan-Fotin and Petrescu, unpublished data). As the number of butterflies that can be found in Bucharest is over 50 (Székely, 2011b, Ștefan-Fotin, Petrescu, unpublished data), it can be assessed that Teilor Park sheltered at least a third of the total butterfly species in Bucharest. 107 plant species belonging to 37 families were identified in the park (Table 1). Most of them are spontaneous plant species. Lavender, but also linden trees, are the main sources of nectar that the park offers during the flowering periods of these species. When the lavender is not in bloom and the spontaneous vegetation has not been mowed, or between mowing series, a multitude of species from the spontaneous flora manage to bloom, mostly attractive to pollinators, including butterflies. Among the plant families, the best represented is Asteraceae, with 20 species, followed by the Fabaceae family, with 13 species (Figure 5). The species of the two families, but not only,

are very important for the food of the adults of many species, and on the flowers of some of them we caught butterflies collecting nectar with their proboscis.
In 2024, three mowing series were applied, between April and July. In July, August and September mowing was carried out more frequently. After mowing, a drastic decrease in butterfly species was observed, but the spontaneous vegetation recovered quite quickly, restoring the biodiversity of insect species (Figure 6, Table 3)
In June 2024, the highest number of butterfly species was recorded, namely 16 species (Figure 6). In July, after mowing around the lavender plants, only 9 species were found, the number of species being in a slight decrease until October when 8 species were recorded, and the mowed vegetation began to recover, temperatures remaining high (Figure 6).

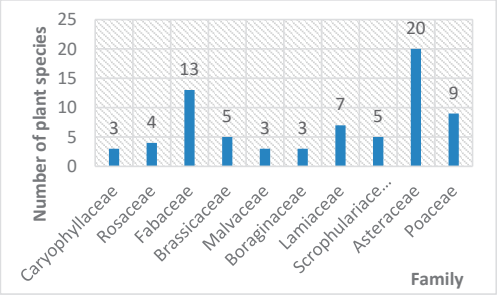


Figure 5. The families with the largest number of plant species in Teilor Park.

In the winter months (January, February, December), no butterflies were observed while flying, although they were days when higher temperatures were recorded. In March, only 4 species were recorded (Figure 6). The first butterflies to appear were *Pieris rapae* and *Pontia edusa*. *Carcharodus alceae* and *Lycaena phlaeas* appeared at the end of the month. In November there were 3 species, respectively *Pieris rapae*, *Pontia edusa* and *Lycaena phlaeas* (Figure 6, Table 4) In November, 2 larvae of *Pieris rapae* were also found (Table 4). *Pieris rapae* was the species that was seen more frequently, with the highest abundance (Table 3, Table 4).
The Hesperiidae family was represented by 3 species: *Carcharodus alceae* (Figure 8, Figure 9), *Erynnis tages* (Figure 7) and *Ochlodes sylvanus* (fig.10) (Table 2). Poaceae such as *Dactylis glomerata* or Poa spp. could be hosts for *Ochlodes sylvanus*; fabaceae such as Trifolium spp. or *Lotus corniculatus* can be consumed by the larvae of the *Erynnis tages*, and *Carcharodus alceae* forages with mallow species (Rákosy, 2024). On 19th May 2024 we observed a larva of *Carcharodus alceae* on a leaf of *Alcaea rosea* (Figure 9).

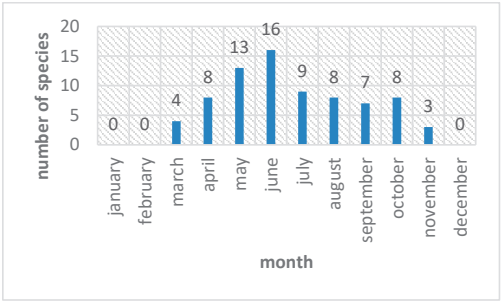


Figure 6. Number of butterfly species monthly observed in Teilor Park in 2024



Figure 7. *Erynnys tages*, feeding on *Lavandula angustifolia*, in Teilor Park

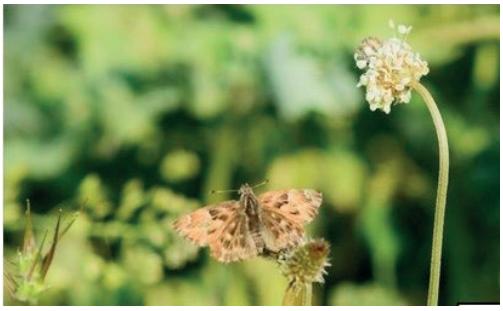


Figure 8. *Carcharodus alceae* on *Plantago lanceolata*, in Teilor Park

The Papilionidae family was represented by the swallowtails *Iphiclides podalirius* (Figure 10, Table 2, Table 4) and *Papilio machaon* (Figure

11, Table 2, Table 4). *Iphiclides podalirius* was observed in all 3 years. We did not identify possible food sources for the caterpillars of this species in the park, but the yards of houses neighboring the park could be a source of shrubs from the Rosaceae family, which are hosts for the larvae of this butterfly species, as mentioned in literature (Rákosy, 2024).



Figure 9. *Carcharodus alceae* catterpillar on the leaf of *Alcaea rosea* in Teilor Park

For *Papilio machaon*, which feeds on Apiaceae species (Rákosy, 2024), *Daucus carota*, the only Apiaceae species identified in the park could be a host. Imago was observed feeding on inflorescences of *Trifolium pratense,* on the lawn near the sports field (Figure 11).
Adults of the 5 species of the Pieridae family were seen between June and October feeding on lavender, but also on plants from the spontaneous flora.
Among the Pieridae, the larvae of Pieris species (*Pieris brassicae*, *P. napi* and *P. rapae*), but also those of *Pontia edusa*, feed on various species of the Brassicaceae family (Rákosy, 2024). *Pieris brassicae* is considered a near threatened species, due to the use of pesticides (Rákosy et al., 2021, Rákosy, 2024, Table 2).



Figure 10. *Iphiclides podalirius* and *Ochlodes sylvanus*, feeding on *Lavandula angustifolia*



Figure 11. *Papilio machaon*, feeding on inflorescences of *Trifolium pratense* in the Teilor Park

The larvae of *Colias croceus* feed on various species of Fabaceae, such as clover and alfalfa (Rákosy, 2024); adults were observed feeding on lavender inflorescences, but also on clovers and *Echium vulgare* (Figure 12, Table 5). Adults of *Pontia edusa* (Figure 15) were seen flying early, starting in March and continuing into November, feeding on various nectar sources (Table 5).



Figure 12. *Colias croceus* in Teilor Park, feeding on inflorescences of *Lavandula angustifolia*

Pieris brassicae (Figure 13) and *Pieris napi* were seen feeding on lavender (Figure 14, Table 5).



Figure 13. *Pieris brassicae* and *Pieris rapae* in the Teilor Park, on lavender

Among the species of the Lycaenidae family, *Lycaena phlaeas* and *Lycaena thersamon* feed, in the larval stage, on species from the Polygonaceae family, such as Rumex spp., and the larvae of the species *Polyommatus icarus*

and *Cupido argiades* consume various fabacee (Rákosy, 2024, Țugulea et al., 2016), being oligofagous species (Țugulea et al., 2016). Adults of the species *Cupido argiades* were seen in the Teilor Park in 2022 and 2024, feeding on *Trifolium repens* and on inflorescences of *Lotus corniculatus*, and each time a single individual was seen (Figure 17, Table 5, Table 6). *Polyommatus icarus* was seen on fabaceae, but also on lavender and other species (Figure 16, Table 5). *Lycaena thersamon* (Figure 19) is a near threatened species at national level, (Rákosy et al., 2021, Rákosy, 2024), with only two observations in the park: in August 2022 and in May 2024 (Table 4, Table 6). *Lycaena phlaeas* was frequently seen in the park, flying and feeding on lavender or on various species of spontaneous flora (Figure 18).



Figure 14. *Pieris napi* feeding on *Lavandula angustifolia* in the Teilor Park



Figure 15. *Pontia edusa*, feeding on inflorescence of *Trifolium pratense* in Teilor Park

On August 29, 2024 we observed an exemplar belonging to the species *Leptotes pirithous* (Figure 18) and in the vicinity an individual of *Lampides boeticus* (Figure 19) feeding on clover inflorescences (Table 4, Table 6). These two species are considered migratory xerothermophilous, and the few individuals that reach Romania probably do not survive the winters (Rákosy, 2024). In recent years, the two species of subtropical origin have appear more frequently in Romania, with more reports in Dobrogea (Dincă et al., 2009, Székely, 2011). After mowing, we did not find either of these two species in the park in 2024.



Figure 16. *Polyommatus icarus*, feeding on flowers of *Lotus corniculatus* in Teilor Park



Figure 17. *Cupido argiades* in Teilor Park, feeding on flowers of *Lotus corniculatus* in Teilor Park



Figure 18. *Lycaena phlaeas* in the Teilor Park, feeding on the inflorescence of *Tripleurospermum inodorum*

Among the Nymphalidae, *Lasiommata megera* and *Vanessa cardui* were observed more frequently (Table 4).

The larvae of the *Vanessa cardui* butterfly are polyphagous, but prefer the leaves of Cirsium species (Rákosy, 2024). In the park, the adult of *Vanessa cardui* was seen feeding on lavender (Figure 23), but also on *Trifolium pratense* (Table 5).

The larvae of *Lasiommata megera* feed on *Dactylis glomerata* and other poaceae (Rákosy, 2024).



Figure 19. *Lycaena thersamon* feeding on flowers of *Berteroa incana* in Teilor Park

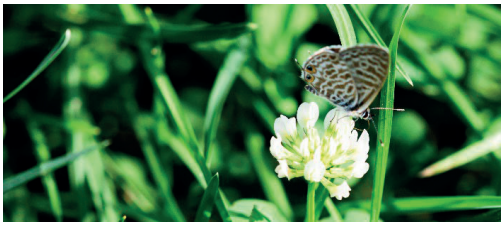


Figure 20. *Leptotes pirithous* in Teilor Park, feeding on inflorescences of *Trifolium repens*

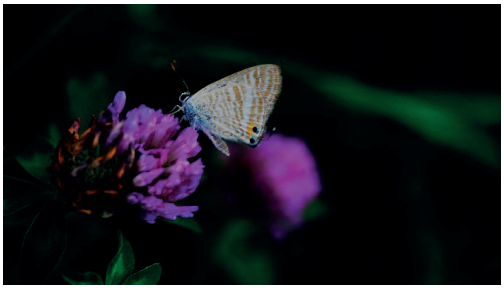


Figure 21. *Lampides boeticus* in the Teilor Park feeding on *Trifolium pratense*

In the park, the adult of *Lasiommata megera* was seen feeding on lavender (Figure 22) and on *Taraxacum officinale* (Table 5).
Vanessa atalanta was observed less frequently, once feeding on the nectar of lime-tree flowers (Figure 25). Nettles, which are food for the larvae of this butterfly (Rákosy, 2024), are not present in the park.
Issoria lathonia (Figure 26) was seen feeding on lavender and red clover (Table 5). The food for the larvae is the *Viola* species (Rákosy, L., 2024), of which we have inventoried the species *Viola tricolor* in the park (Table 1). *Melitaea phoebe* was observed only on May 6, 2024 (Table 4, Table 6), feeding on red clover inflorescences (Figure 24, Table 5).
Lavender provides nectar for the butterfly species in the park, during the flowering period, June-October; 15 of the 21 butterfly species observed in the park were seen feeding on lavender (Table 5).



Figure 22. *Lassimmata megera*, feeding on lavender Teilor Park

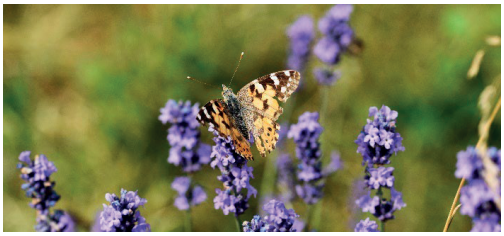


Figure 23. *Vanessa cardui*, in the Teilor Park, feeding on inflorescences of lavender

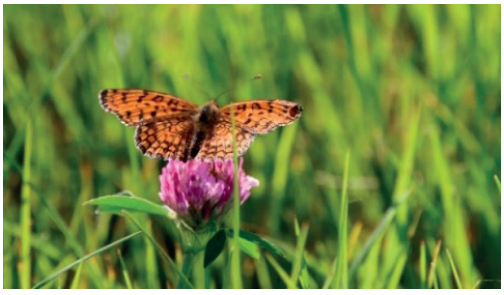


Figure 24. *Melitaea phoebe* in Teilor Park

Beside lavender, butterflies were seen feeding on Tilia spp. and on 23 species of spontaneous flora; 12 species of butterflies were captured feeding on *Trifolium pratense* (Table 5). During the period when lavender is not in bloom, food for adults can be provided by spontaneous species such as *Taraxacum officinale*, *Tripleurospermum inodorum*, *Buglossoides arvensis* and others (Table 5).



Figure 25. *Vanessa atalanta* on linden in the Teilor Park

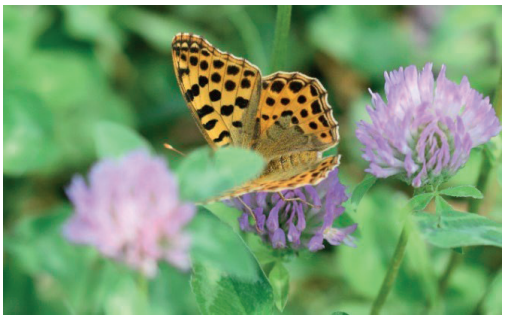


Fig 26. *Issoria lathonia* on inflorescences of *Trifolium pratense* in Teilor Park

Table 1 The list of the plants in the Teilor Park, 2022-2024

	Family	Species
1.	Ranunculaceae	*Ranunculus sardous, Ranuculus ficaria.*
2.	Papaveraceae	*Papaver rhoeas, Papaver dubium*
3.	Betulaceae	*Betula pendula**
4.	Juglandaceae	*Juglans regia*
5.	Portulacaceae	*Portulaca oleracea*
6.	Caryophyllaceae	*Silene latifolia, Stellaria media, Gypsophila muralis*
7.	Amaranthaceae	*Amaranthus retroflexus*
8.	Chenopodiaceae	*Chenopodium hybridum, Atriplex tatarica*
9.	Polygonaceae	*Polygonum aviculare, Rumex* spp..
10.	Rosaceae	*Potentilla argentea, Potentilla reptans, Rosa canina, Rubus caesius*.
11.	Fabaceae	*Coronilla varia, Lathyrus tuberosus, Lotus corniculatus, Medicago sativa, Medicago lupulina, Melilotus alba, Melilotus officinalis* Pall., *Trifolium fragiferum, Trifolium pratense, Trifolium repens, Vicia cracca, Vicia grandiflora, Vicia hirsuta,*
12.	Oxalidaceae	*Oxalis dilenii*.
13.	Geraniaceae	*Erodium cicutarium*, *Geranium dissectum*.
14.	Zygophyllaceae	*Tribulus terrestris.*
15.	Vitaceae	*Parthenocissus inserta*
16.	Apiaceae	*Daucus carota.*
17.	Hypericaceae	*Hypericum perforatum*.
18.	Violaceae	*Viola tricolor*.
19.	Brassicaceae	*Berteroa incana, Capsella bursa-pastoris, Cardaria draba, Erophila verna (Draba verna), Rorippa austriaca*
20.	Salicaceae	*Salix sp.**
21.	Tiliaceae	*Tilia sp.**
22.	Malvaceae	*Alcea rosea, Hibiscus syriacus*, Malva sylvestris*.
23.	Primulaceae	*Anagallis arvensis* L.
24.	Rubiaceae	*Galium aparine, Galium humifusum.*
25.	Valerianaceae	*Valerianella locusta*.
26.	Dipsacaceae	*Dipsacus fullonum.*
27.	Convolvulaceae	*Calystegia sepium, Convolvulus arvensis.*
28.	Boraginaceae	*Asperugo procumbens, Buglossoides arvensis, Echium vulgare.*
29.	Verbenaceae	*Verbena officinalis*.
30.	Lamiaceae	*Ajuga genevensis, Ballota nigra, Glechoma hederacea, Lamium amplexicaule, Lamium purpureum, Lavandula angustifolia**Mill., *Mentha longifolia*.
31.	Solanaceae	*Solanum nigrum*
32.	Scrophulariaceae	*Linaria vulgaris, Verbascum spp., Veronica hederifolia, Veronica persica, Veronica polita*.
33.	Plantaginaceae	*Plantago lanceolata*
34.	Asteraceae	*Achillea sp., Ambrosia artemisiifolia, Artemisia absinthium, Anthemis austriaca, Artemisia vulgaris, Carduus acanthoides, Centaurea cyanus, Cirsium arvense, Cirsium vulgare, Chondrilla juncea, Crepis spp., Erigeron annuus, Inula britannica, Lactuca serriola, Matricaria chamomilla, Onopordum acanthium, Senecio vulgaris, Sonchus arvensis, Taraxacum officinale, Tripleurospermum inodorum*
35.	Poaceae	*Cynodon dactylon, Dactylis glomerata, Echinochloa crus-galli, Festuca sp., Hordeum murinum, Lolium spp., Poa spp., Sorghum halepense., Phragmites australis*
36.	Juncaceae	*Juncus sp.*
37.	Cupressaceae	*Juniperus* sp., Metasequoia* sp.*

Legend: * Plant grown in the park for ornamental purposes

Table 2. Species of butterflies observed in the Teilor Park in 2022-2024

No.	Family	No.	Species	year			Threat category (Rákosy et al., 2021, Rákosy 2024)
				2022	2023	2024	
1.	Hesperiidae	1.	*Carcharodus alceae* (Esper, 1780)	-	+	+	LC
		2.	*Erynnis tages* (Linnaeus, 1758)	-	-	+	LC
		3.	*Ochlodes sylvanus* (Esper, 1777)	+	-	+	LC
2.	Papilionidae	4.	*Iphiclides podalirius* (Linnaeus, 1758)	+	+	+	LC
		5.	*Papilio machaon* (Linnaeus, 1758)	-	-	+	LC
3.	Pieridae	6.	*Colias croceus* (Geoffroy, 1785)	+	+	+	LC
		7.	*Pieris brassicae* (Linnaeus 1758)	-	-	+	NT
		8.	*Pieris napi* (Linnaeus, 1758)	+	+	+	LC
		9.	*Pieris rapae* (Linnaeus, 1758)	+	+	+	LC
		10.	*Pontia edusa* (Fabricius, 1777)	+	+	+	LC
4.	Lycaenidae	11.	*Cupido argiades* (Pallas, 1771)	+	-	+	LC
		12.	*Lampides boeticus* (Linnaeus, 1767)	-	-	+	NA
		13.	*Leptotes pirithous* (Linnaeus, 1767)	-	-	+	NA
		14.	*Lycaena phlaeas* (Linnaeus, 1761)	+	+	+	LC
		15.	*Lycaena thersamon* (Esper, 1784)	+	-	+	NT
		16.	*Polyommatus icarus* (Rottemburg, 1775)	+	+	+	LC
5.	Nymphalidae	17.	*Issoria lathonia* (Linnaeus, 1758)	-	-	+	LC
		18.	*Lasiommata megera* (Linnaeus, 1767)	-	+	+	LC
		19.	*Melitaea phoebe* (Denis & Schifermüller 1775)	-	-	+	LC
		20.	*Vanessa atalanta* (Linnaeus, 1758)	+	-	+	LC
		21.	*Vanessa cardui* (Linnaeus, 1758)	+	+	+	LC
TOTAL SPECIES OBSERVED				12	10	21	

Legend: LC - low concern; NT - near threatened; NA - non - autochthonous and migratory species

Table 3 Number of individuals monthly recordings on transects in 2024

No.	Species	data								
		16th march	14th april	18th may	10th june	7th july	29th august	8th september	13th october	2th november
1.	*Carcharodus alceae*	0	0	0	1	0	3	4	0	0
2.	*Iphiclides podalirius*	0	1	0	0	5	0	0	0	0
3.	*Colias croceus*	0	0	0	0	4	4	3	0	0
4.	*Pieris brassicae*	0	0	3	4	0	0	0	6	0
5.	*Pieris napi*	0	0	0	2	0	0	0	0	0
6.	*Pieris rapae*	9	12	14	22	11	10	8	7	3
7.	*Pontia edusa*	4	3	5	6	0	0	0	4	1
8.	*Lycaena phlaeas*	0	2	2	5	4	4	3	0	2
9.	*Polyommatus icarus*	0	0	0	1	5	4	5	8	0
10.	*Issoria lathonia*	0	0	0	1	1	0	0	0	0
11.	*Vanessa cardui*	0	0	0	0	0	0	1	0	0
	Number of species	2	4	5	8	6	6	5	4	3
	Number of individuals	13	18	26	46	30	26	23	25	6

Table 4. Recordings of butterfly's species in Teilor Park (2022-2024)

No.	Species	Recordings	
		year	day/month
1.	*Carcharodus alceae*	2023	09/04.
		2024	31/03, 12/04, 21/04, 27/04, 28/04, 01/05, 19/05-L, 3/06, 07/06, 10/06., 12/06, 22/06, 20/07, 29/08, 08/09, 15/09.
2.	*Erynnis tages*	2024	12/04, 22/06, 30/06.
3.	*Ochlodes sylvanus*	2022	07/08, 20/08.
		2024	26/05, 02/06, 07/06.
4.	*Iphiclides podalirius*	2022	07/08, 12/08.
		2023	09/04, 09/07.
		2024	14/04, 07/06, 12/06, 30/06., 07/07, 20/07, 15/09, 15/10
5.	*Papilio machaon*	2024	07/06, 07/07.
6.	*Colias croceus*	2022	15/07, 03/11.
		2023	09/07.
		2024	21/04, 01/05, 07/06, 30/06, 07/07, 13/07, 20/07, 29/08, 08/09, 21/09, 28/09.
7.	*Pieris brassicae*	2024	02/06, 3/06, 07/06, 13/10.
8.	*Pieris napi*	2022	9/10.
		2023	25/03.
		2024	26/05, 02/06.
9.	*Pieris rapae*	2022	07/08, 12/08, 20/08, 09/10, 23/10, 03/11, 09/03.
		2023	05/03, 12/03, 25/03, 20/05, 09/07, 08/10, 15/10.
		2024	10/03, 16/03, 30/03, 31/03, 14/04, 21/04, 27/04, 28/04, 01/05, 04/05, 05/05, 10/05, 12/05, 18/05, 19/05, 26/05, 02/06, 3.06, 07/06, 30/06, 07/07, 20/07.2024, 29/08, 08/09, 28/09, 06/10, 13/10, 22/10, 02/11, 16.11 (2-L)
10.	*Pontia edusa*	2022	19/09.
		2023	05/03, 12/03.
		2024	03/03, 10/03, 16/03, 14/04, 01/05 (A+P), 04/05, 10/05, 12/05, 18/05, 24/05, 26/05, 3/06, 08/09, 28/09, 13/10.
11.	*Cupido argiades*	2022	15/07
		2024	07/07
12.	*Lampides boeticus*	2024	29/08.
13.	*Leptotes pirithous*	2024	29/08.
14.	*Lycaena phlaeas*	2022	20/08, 09/10, 03/11.
		2023	27/08, 15/10, 21/10, 22/10, 29/10
		2024	30/03, 14/04, 04/05, 05/05, 10/05, 12/05, 18/05, 07/06, 22/06, 30/06, 07/07, 13/07, 20/07, 29/08, 08/09, 06/10, 13/10.
15.	*Lycaena thersamon*	2022	07/08.
		2024	12/05.
16.	*Polyommatus icarus*	2022	15/07, 07/08, 20/08, 09/10.
		2023	09/07, 07/08, 15/10, 21/10.
		2024	04/05, 12/05, 22/06, 30/06, 02/07, 07/07, 13/07, 20/07, 29/08, 08/09, 15/09, 28/09, 06/10, 13/10.
17.	*Issoria lathonia*	2024	10/06, 16/06, 17/06, 07/07.
18.	*Lasiommata megera*	2023	09/07.
		2024	27/04, 28/04, 04/05, 05/05, 18/05, 26/05, 10/06., 12/06, 30/06, 02/07, 28/09, 13/10.
19.	*Melitaea phoebe*	2024	06/05.
20.	*Vanessa atalanta*	2022	15/07.
		2024	07/06, 06/10.
21.	*Vanessa cardui*	2022	12/08, 20/08.
		2023	22/10.
		2024	02/06., 07/06, 29/08.

Legend: A - adult; L - larva; P - pupa

Table 5. The interaction of butterflies in the Teilor Park with the plants that provide nectar for the adult butterflies

Butterfly species	Plant species																								
	Potentilla argentea	Lotus comiculatus	Trifolium pratense	Trifolium repens	Erodium cicutarium	Daucus carota	Berteroa incana	Rorippa austriaca	Tilia sp.	Malva sylvestris	Echium vulgare	Buglossoides arvensis	Verbena officinalis	Convolvulus arvensis	Ajuga genevensis	Lavandula angustifolia	Veronica persica	Plantago lanceolata	Achillea sp.	Cirsium arvense	Carduus acanthoides	Erigeron annuus	Inula britannica	Taraxacum officinale	Tripleurospermum
Carcharodus alceae			+							+	+	+			+	+									
Erynnis tages																+		+							
Ochlodes sylvanus																+									
Iphiclides podalirius			+						+							+					+				
Papilio machaon			+													+									
Colias croceus			+	+							+					+									
Pieris brassicae																+									
Pieris napi																+									
Pieris rapae			+		+					+		+		+		+							+	+	
Pontia edusa	+		+			+		+		+		+	+			+	+	+	+	+				+	+
Cupido argiades		+		+																					
Lampides boeticus			+																						
Leptotes pirithous				+																					
Lycaena phlaeas			+	+												+						+			+
Lycaena thersamon							+																		+
Polyommatus icarus		+	+				+									+							+		
Issoria lathonia			+													+									
Lasiommata megera																+								+	
Melitaea phoebe			+																						
Vanessa atalanta									+																
Vanessa cardui			+													+									

Table 6. Geographic positioning of butterflies with just a few observations in the park (2024)

Species	2024	Geographic coordinates	
		N	E
Cupido argiades	7th july	44.418317	26.188.627
Lampides boeticus	29th august	44.419348	26.188325
Leptotes pirithous	29th august	44.419405	26.188387
Lycaena thersamon	12th mai	44.418424	26.187630
Melithaea phoebe	6th mai	44.420447	26.188629
Papilio machaon	7th june	44.418363	26.187714
	7th july	44.418524	26.188167

CONCLUSIONS

Between July 2022 and November 2024, adults of 21 butterfly species were observed in Teilor Park; on several occasions, other stages, such as larvae and pupa were observed.

Of the butterfly species identified in the park, two species, *Pieris brassicae* and *Lycaena thersamon*, are considered near threatened (NT) at national level.

Pieris rapae was the most abundant species in the park.

In 2024, two migratory and xerothermophilous elements were observed in the park, namely *Leptotes pirithous* and *Lampides boeticus*.

The spontaneous flora, consisting of 99 plant species, could provide food for the larvae of at least 16 butterfly species out of the 21 observed in 2024.

Lavender provides nectar for the adults of the park's butterfly species during the flowering period, May-October; 15 of the 21 species of butterflies observed in the park were seen feeding on lavender.

Beside lavender, butterflies were seen feeding on 23 species of spontaneous flora. 12 species of butterflies were caught feeding on *Trifolium pratense*.

During the study period (2022-2024), through its floristic composition, the park could support relatively well the diversity of butterfly species.

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FRACTAL ANALYSIS IN THE COMPARATIVE STUDY OF *GINKGO BILOBA* L. TREES

Cristian BERAR[1], Cristina TOTA[1], Florin SALA[1, 2]

[1]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, 300645, Timişoara, Romania
[2]Agricultural Research and Development Station Lovrin, Lovrin, 307250, Cluj, Romania

Corresponding author email: florin_sala@usvt.ro

Abstract

The study aimed to detect female and male plants of the species Ginkgo biloba L. based on the fractal properties of leaf geometry. Leaf samples (40 leaves) were taken from female and male tree specimens, from different locations (Timisoara and Deva). To obtain representative samples, by leaf size category, samples were taken randomly, in autumn after leaf fall, on the leaf carpet on the ground, under the crown projection. The box-counting method was used to analyze the leaf geometry and obtain the values of fractal dimensions (D). The fractal analysis led to the fractal dimensions (D), denoted D(F1), D(F2) for female tree specimens and D(M1), D(M2) for male tree specimens. The mean values of the fractal dimensions were D(F1) =1.6737±0.0069, D(F2) =1.5842±0.0125, D(M1) =1.4947±0.0107, D(M2) =1.5146±0.0051. Each sample D(F1), D(F2) was analyzed compared to each sample D(M1), D(M2). Comparative analysis of fractal dimension values (means, medians) from female and male specimens led to statistically significant differences ($p<0.001$). Fractal analysis was a reliable method for detecting the two categories of trees, female and male, under the study conditions.

Key words: *box-counting, comparative analysis, fractal dimension, ginkgo leaves, hierarchical clustering.*

INTRODUCTION

Ginkgo biloba L. (Ginkgoaceae, Ginkgoales), is a dioecious, deciduous tree species native to southeastern China (Cheng & Fu, 1978; Lin et al., 2022). Ginkgo has high ecological plasticity, with adaptability to very varied climatic conditions (Lin et al., 2022).

Ginkgo has high socio-cultural value for several nations, along with important ecological and economic benefits (Crane, 2019; Lin et al., 2022; Lee et al., 2023).

Ginkgo has been referred to as a plant with numerous "compounds with unique structures", which resulted from its distinct biological characteristics (Liu et al., 2022). *Ginkgo biloba* contains a series of active principles of importance for the pharmaceutical, medical, cosmetic, and food industries (Liu et al., 2022; Noor-E-Tabassum et al., 2022; Akaberi et al., 2023). Favorable effects of some preparations and products containing active ginkgo principles have been recorded in the pharmacological and medical fields (Akaberi et al., 2023).

Ginkgo biloba has been found to be a species with very good adaptability to the urban environment, to stress factors specific to the urban environment, and ginkgo trees are present in various parks and green spaces in the urban environment (Cui et al., 2022; Lin et al., 2022; Kisvarga et al., 2024).

Fossilized ginkgo leaves have been studied in relation to factors and conditions specific to the geological eras in which they formed and grew (Sun et al., 2003). The comparative analysis of some ginkgo genotypes was made based on foliar parameters (Klimko et al., 2015).

Different morphological types of leaves have been identified in ginkgo, as a different expression of the genes involved and the molecular mechanisms (Tang et al., 2022).

Different leaf types and leaf modifications have been identified and reported in ginkgo, with variable frequency, higher in the normal form (Li et al., 2024). It was confirmed that morphological differentiation of leaves in ginkgo occurred at the level of leaf primordia, through gene expression and specific molecular processes (Li et al., 2024).

Various methods of analysis, microscopic, geometric, topological, have been used to analyze and study ginkgo leaves (Carvalho et al., 2017; Hang et al., 2021). Based on the

results, Hang et al. (2021) identified certain distinctive elements, specific to the studied leaves, which led to the differentiation of ginkgo leaf shape.
Early differentiation of female and male specimens in ginkgo seedlings is important for the selection of seedlings in relation to the planting destination, the purpose for which they are cultivated, and different "ginkgo industries" (Gao et al., 2024). Some differences in ginkgo seedlings were recorded, for their growth capacity and rate (higher in male specimens), and for their content of bioactive compounds (higher in female specimens) (Gao et al., 2024). According to the authors, the results presented interest for a base of indicators in the early selection of female and male specimens in ginkgo (Gao et al., 2024).
The male forms of ginkgo presented certain advantages for practice, and were of interest for landscaping, and the female forms for fruit production (Fu et al., 2021; Li et al., 2023).
Electrochemical techniques (peroxidase content detection) and molecular techniques (male-specific SCAR gene) were used to identify and select female and male ginkgo forms (Fu et al., 2021; Lee et al., 2023).
This research attempted to differentiate female and male forms in mature ginkgo trees, based on leaf geometry expressed in fractal dimension (D).

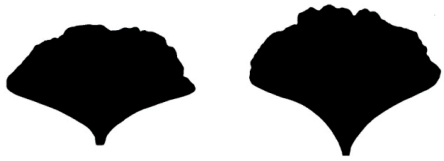
MATERIALS AND METHODS

For the comparative analysis of female and male specimens of *Ginkgo biloba* L. trees, the leaves were used as biological material. Leaf samples were taken in autumn from the carpet of fallen leaves on the ground (Figure 1).



Figure 1. Leaf mat, *Ginkgo biloba* L. species; base for collecting leaf samples at variable distances from the stem

On the leaf mat, on the ground, the leaves came from different areas of the crown, and in this way a much more homogeneous sampling base was ensured. Leaf samples, of variable sizes, were taken randomly, at different distances from the tree trunk. 40 leaves were taken for each sample.
Two female specimens of ginkgo trees from Timisoara (F1) and Deva (F2), and two male specimens of ginkgo trees from Timisoara (M1, M2), were considered. In all cases, the samples were collected from mature trees.
On the leaf litter, the leaves came from different areas of the tree crown. Thus, the sampling base had a high degree of homogeneity. Leaf samples of different sizes were taken from several points on the leaf litter below the crown projection.
Each leaf was scanned at 1:1 scale (HP, CM2320fxi MFP, fixed scanner). The digital images, binarized (Figure 2), were analyzed to obtain the fractal geometry parameters of the leaves. The foreground pixels (FP) values and the fractal dimension (D) were recorded. Box-counting analysis was used (Voss, 1985), and ImageJ (Rasband, 1997).



(a) (b)

Figure 2. Examples of scanned leaves, *Ginkgo biloba* L. species; (a) female specimen leaf, (b) male specimen leaf; images in binarized format

The overall evaluation of the data series was done through descriptive statistical analysis, and the reliability of the data was done through the Anova test. The interdependence relationship between fractal dimensions (D) and foreground pixels (FP) was evaluated by regression analysis. The comparison between leaf samples from female specimens, D(F1), D(F2) and male specimens (D(M1), D(M2) was made on the basis of fractal dimension, mean values (t-test), and median values (Wilcoxon test). Established statistical confidence parameters were used to assess the reliability of the results (p, and RMSE). The mathematical

analysis on the fractal parameters in ginkgo leaves was made in EXCEL, and in the PAST software (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

Ginkgo leaves, in total 160 leaves, collected from the two categories, in two repetition foe each category (40 from each category), two female specimens (F1, F2) and two male categories (M1, M2) were analyzed to describe the fractal geometry of the leaves. Values were obtained for the foreground pixels (FP) parameter and for the fractal dimension (D) for each sample category.

Descriptive statistical analysis generated the synthetic values in Table 1. According to the Anova Test (95% confidence), the results statistical safety was confirmed (Table 2). The presence of variance was also confirmed, as shown in Table 2.

Table 1. Descriptive statistical analysis report, *Ginkgo biloba* leaf samples, female and male specimens

Statistical parameters	Fractals parameters							
	Female tree specimens				Male tree specimens			
	F1 samples		F2 samples		M1 samples		M2 samples	
	FP(F1)	D(F1)	FP(F2)	D(F2)	FP(M1)	D(M1)	FP(M2)	D(M2)
N	40	40	40	40	40	40	40	40
Min	66603	1.5839	95033	1.4309	53769	1.3270	97666	1.4279
Max	174685	1.7445	396132	1.7601	234369	1.5983	225410	1.5707
Sum	4783749	66.9499	8666929	63.3693	5540525	59.4999	6321522	60.5840
Mean	119593.7	1.6737	216673.2	1.5842	138513.1	1.4875	158038	1.5146
Std. error	4091.015	0.0069	11733.08	0.0125	6422.454	0.0090	4650.453	0.0051
Variance	6.69E+08	0.001926	5.51E+09	0.006237	1.65E+09	0.00321	8.65E+08	0.001046
Stand. dev	25873.85	0.043884	74206.51	0.078976	40619.17	0.056658	29412.05	0.032339
Median	120178	1.6738	210613	1.5828	139661	1.4979	155011	1.5154
25 percentile	98701.75	1.64825	154177	1.5228	111353.3	1.4500	138858	1.49515
75 percentile	140103.5	1.711325	253433.3	1.625175	171215.3	1.5330	182144.8	1.539425
Skewness	0.0567	-0.4375	0.5474	0.4294	0.1142	-0.6809	0.1379	-0.5522
Kurtosis	-0.6919	-0.4640	-0.0317	-0.1727	-0.2385	0.6367	-0.1924	0.2047
Geom. mean	116769.10	1.67	204307.50	1.58	132208.30	1.49	155312.20	1.51
Coeff. var	21.63	2.62	34.25	4.99	29.33	3.81	18.61	2.14

Table 2. Summary of the Anova Test

Source of Variation	SS	df	MS	F	P value	F crit
Between Groups	2.21E+12	7	3.16E+11	291.1555	1E-132	2.0390
Within Groups	3.39E+11	312	1.09E+09			
Total	2.55E+12	319				

The series of fractal dimension data, D(F1), D(F2), D(M1), and D(M2) presented a normal distribution, with r = 0.9804 (D(M1) to r = 0.9858 (D(M2) (Figure 3).

The FP parameter showed low variability in the FP(M2) data series, CV = 18.61, medium variability in the FP(F1) data series, CV = 21.63, and in the FP(M1) data series, CV = 29.33. High variability was recorded in the FP(F2) data series, CV = 34.25.

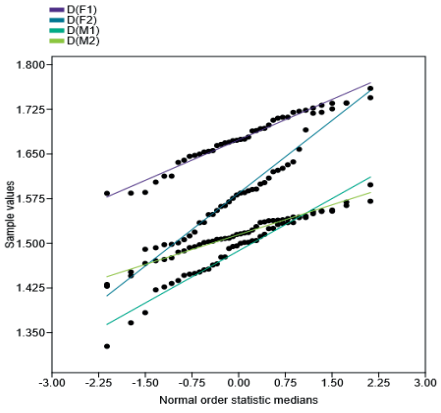


Figure 3. Distribution of D values

In the case of fractal dimension (D), low variability was recorded in all data series, CV =

2.14 (D(M2) to CV = 4.99 (D(F2).
The variation of fractal dimension D was analyzed in relation to the values of the FP parameter, for each leaf sample.
The series of D(F1) values varied in relation to FP(F1) according to equation (1), $R^2 = 0.635$, F = 34.87, $p<0.001$ (Figure 4).
The series of D(F2) values varied in relation to FP(F2) according to equation (2), $R^2 = 0.562$, F = 23.871, $p<0.001$.
The series of D(M1) values varied in relation to FP(M1) according to equation (3), $R^2 = 0.986$, F = 1327.3, $p<0.001$ (Figure 5).
The series of D(M2) values varied in relation to FP(M2) according to equation (4), $R^2 = 0.973$, F = 660.23, $p<0.001$.

$$D(F1) = -6.61E-12x^2 + 2.948E-06x + 1.42 \quad (1)$$

where: x – FP(F1)

$$D(F2) = -2.308E-12x^2 + 1.859E-06x + 1.302 \quad (2)$$

where: x – FP(F2)

$$D(M1) = -4.971E-12x^2 + 2.761E-06x + 1.208 \quad (3)$$

where: x – FP(M1)

$$D(M2) = -4.933E-12x^2 + 2.647E-06x + 1.224 \quad (4)$$

where: x – FP(M2)

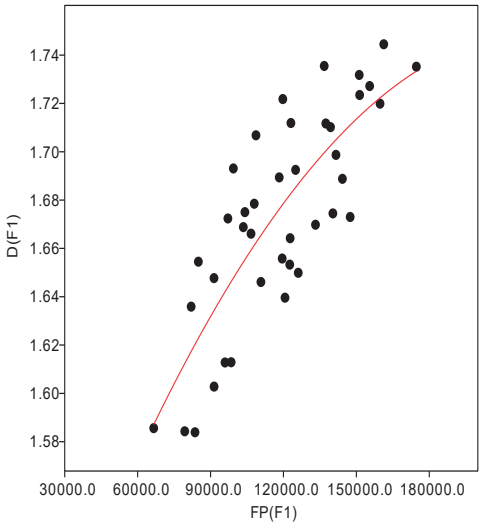


Figure 4. Variation of D(F1) in relation to FP(F1)

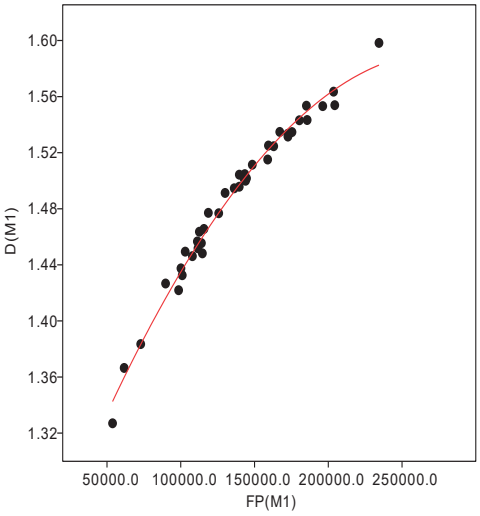


Figure 5. Variation of D(M1) in relation to FP(M1)

From the analysis of the values of the regression coefficient (R^2), the parameter F, equations (1) – (4), as well as the graphical distribution (Figures 4 and 5), a much closer (stronger) relationship was observed between the parameter D and FP in the case of leaf samples from male trees (DM), compared to the situation of leaf samples from female trees (DF).
RMSE values confirmed the high reliability of the D and FP relationship in the case of leaf samples from ginkgo male specimens, compared to female ones; RMSE = 0.0255 for D(F1)–FP(F1) relationship, RMSE = 0.0516 for D(F2)–FP(F2) relationship, RMSE = 0.0066 for D(M1)–FP(M1) relationship, and RMSE = 0.0053 for D(M2)–FP(M2) relationship.
Comparative analysis of leaf samples from female Ginkgo tree specimens was done, based on fractal dimensions (D), in relation to each leaf sample from male tree specimens.
From the analysis of the series of fractal dimensions D(F1), associated with leaves from female tree specimens, with the fractal dimensions from male specimens, D(M1), and D(M2), the statistical values of the applied tests resulted (Table 3).
The mean value, and the median, in the case of D(F1) were higher compared to the mean and median values of D(M1), and D(M2), $p<0.001$ (Table 3).

Table 3. Comparative analysis results, D(F1) samples compared to D(M1), D(M2) leaf samples

Statistical parameters	Test values compared to D(M)	
	D(M1)	D(M2)
Given mean: D(F1)	1.6737	1.6737
Sample mean: D(M)	1.4875	1.5146
95% conf. interval:	(1.4694 1.5056)	(1.5043 1.5249)
Difference:	0.1862	0.1591
95% conf. interval:	(0.16808 0.20432)	(0.14876 0.16944)
t :	-20.785	-31.115
p (same mean):	1.05E-22	3.78E-29
Significance of mean differences	***	***
Given median: D(F1)	1.6738	1.6738
Sample median: D(M)	1.4979	1.5153
W :	820	820
Normal appr. z :	5.5109	5.5109
p (same median):	3.57E-08	3.57E-08
Significance of median differences	***	***

In the case of fractal dimensions D(F2), associated with leaf samples from female tree specimens, analyzed in comparison with fractal dimensions from male specimens, D(M1), and D(M2), the values of the applied tests resulted (Table 4). The mean value and median in the case of D(F2) were higher compared to the mean and median values of D(M1), and D(M2), $p<0.001$.

Table 4. Comparative analysis results, D(F2) samples compared to D(M1), D(M2) leaf samples

Statistical parameters	Test values compared to D(M)	
	D(M1)	D(M2)
Given mean: D(F2)	1.5842	1.5842
Sample mean: (D(M)	1.4875	1.5146
95% conf. interval:	(1.4694 1.5056)	(1.5043 1.5249)
Difference:	0.096702	0.0696
95% conf. interval:	(0.078582 0.11482)	(0.059257 0.079943)
t :	-10.795	-13.612
p (same mean):	2.81E-13	2.13E-16
Means are significantly different	***	***
Given median: D(F2)	1.5828	1.5828
Sample median: D(M)	1.4979	1.5153
W :	819	820
Normal appr. z :	5.4975	5.5109
p (same median):	3.85E-08	3.57E-08
Medians are significantly different	***	***

The mean and median values of fractal dimensions (D), and the differences calculated for leaf samples from female specimens D(F1), D(F2), compared to male specimens D(M1), D(M2), are presented in Figure 6 for the mean values, and in Figure 7 for the median values.

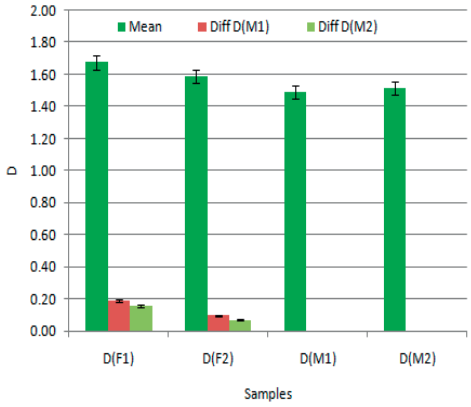


Figure 5. The differences calculated in the case of mean values of fractal dimensions

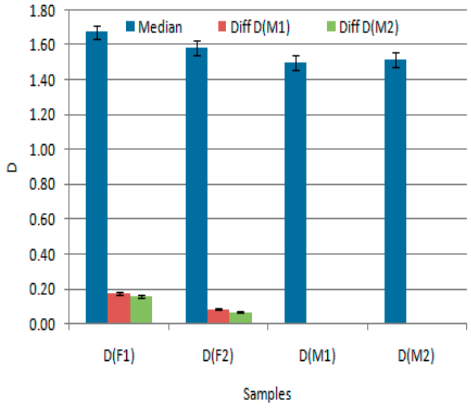


Figure 6. The differences calculated in the case of median values of fractal dimensions

The degree of similarity between the fractal dimensions of male specimens, D(M1), D(M2) was at the level of SDI = 0.4014. The similarity level between D(M1) and D(F1) was SDI = 1.2832, and between D(M1) and D(F2) was SDI = 0.7900. The level of similarity between D(M2) and D(F1) was SDI = 1.0576, and between D(M2) and D(F2) was SDI = 0.6907. In all cases, the level of similarity, based on D values, was lowest between male forms, and

highest in the comparative relationship between male and female forms.
The four leaf types were positioned in distinct clusters, D(F1) with D(F2), and D(M1) with D(M2) within the cluster diagram (Coph.corr. = 0.683), hierarchical clustering (Figure 7).

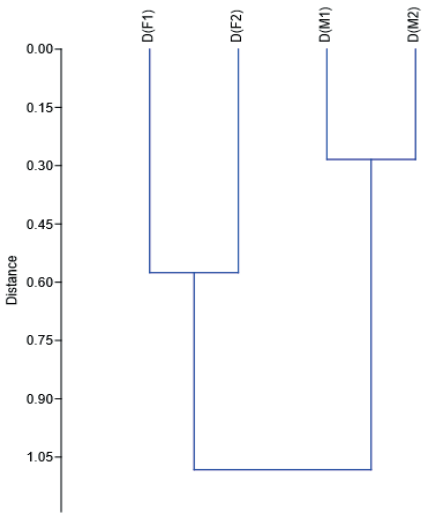


Figure 7. Cluster arrangement of fractal dimensions in female and male forms, ginkgo leaves

Studies on the leaves of trees and shrubs have analyzed anatomical, morphological, and physiological aspects, as well as comparative analyses, classification methods, and responses to vegetation and environmental stress factors, etc. (H'ng & Loh, 2018; Delian and Săvulescu, 2023; Delian et al., 2024; Săvulescu et al., 2024).
Leaves from different ginkgo genotypes were studied and differentiated based on morphological parameters, and obvious differentiations were recorded based on leaf and petiole sizes (Klimko et al., 2015). Leaf and petiole dimensions, as well as certain elements at the "adaxial/abaxial" level, were considered highly specific parameters, useful for differentiating ginkgo leaves from the tested genotypes (Klimko et al., 2015).
Through molecular tests of a collection of 42 ginkgo samples, female and male specimens were identified in a 1:1 ratio. Differentiating female and male forms of ginkgo was of interest for the optimal use of seedlings (Li et al., 2023).
Multivariate analysis (PCA) provided a certain level of separation between female and male ginkgo forms based on tree growth parameters, but there were no differentiations based on wood properties (Li et al., 2023).
The differentiation of leaf shape in ginkgo was recorded based on specific elements, through geometric and topological analysis methods (Hang et al., 2021). Distinctly different values of fractal dimension have been reported in ginkgo in relation to leaf shape (Vlcek & Cheung, 1986). Fractal analysis was applied to highlight certain similarities and specific fractal characteristics with ginkgo leaves (Malischewsky, 2014).
Fractal analysis has captured significant differences in leaf shape in studies of different species of tree plants, or cultivated plants (Vlcek & Cheung, 1986; Sala et al., 2017; Agapie et al., 2020).
To characterize ginkgo trees, in relation to other species, based on laser images, fractal analysis provided significant differentiations (Zhang et al., 2021).
In the case of this research, on leaf samples from mature ginkgo trees, it was possible to differentiate female and male forms, based on the fractal dimension of the leaf geometry.
There is possible variability in the shape of ginkgo leaves in relation to the age of the trees, and this approach, to the fractal geometry of the leaves, requires investigation in comparative studies for clarification, and continued research.

CONCLUSIONS

Fractal analysis described the geometry of ginkgo leaves through fractal dimension values, denoted D(F) for female specimens, and D(M) for male specimens.
The average values of fractal dimensions in female ginkgo leaves, D(F), were higher compared to the fractal dimensions in male leaves, D(M).
The comparative analysis between the two categories of samples, female specimens D(F1), D(F2), and male specimens D(M1), D(M2), led to significant differences for the mean and median values.
A stronger relationship was recorded between the fractal dimension D, and the FP parameter in the case of leaves in male specimens,

compared to female specimens (R^2 = 0.973 in the relationship D(M1) with (FP(M1); R^2 = 0.635 in the relationship D(F1) with FP(F1)).
The results recorded were eloquent for leaf samples from mature ginkgo trees, but require verification in young specimens, where the differentiation of female and male forms is of interest for the differentiated use of seedlings.
In the case of ginkgo seedlings, the accuracy of the method may be lower, due to the lower number of leaves. It is recommended to test this method together with other more accurate but also more expensive methods (e.g. molecular, genetic), for verification and calibration, with the aim of further promotion on a larger scale.

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STUDIES ON THE DEVELOPMENT OF A PAULOWNIA PLANTATION IN ROMANIA

Roberto Renato BERNARDIS[1], Adrian NISTOR[1], Daniela Sabina POȘTA[2]

[1]"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Timişoara, Romania

Corresponding author emails: roberto.bernardis@iuls.ro, danielaposta@usvt.ro

Abstract

Paulownia is a remarkable tree, known for its rapid growth, being one of the most promising horticultural species in Romania. With impressive development, this plant can reach commercial maturity in just 3-6 years, provided that certain essential factors are respected, such as pedological, agrochemical, and agrotechnical conditions, along with specific maintenance work. In a detailed study, two hybrid varieties of Paulownia were analyzed: 'Cotevisa 2' and 'Superhibrid Z07F1'. These are cultivated in the Paulownia Sipote plantation, located in the picturesque village of Sipote, in Iași County. The plantation was established in 2021, which is also when the measurements began. During the first four years of development, the establishment and maintenance work, as well as the growth of these varieties, were closely monitored. Rigorous statistical calculations were performed to evaluate progress and identify the factors contributing to the successful cultivation of Paulownia in Romania. The main goal is to collect and analyze valuable data to support farmers in the efficient cultivation of this promising tree.

Key words: *Paulownia, tree, variety, irrigation, diameter, experimental variants.*

INTRODUCTION

Paulownia, also known as the "princess tree", is a fascinating presence in the botanical world, having been cultivated for thousands of years and revered in Chinese culture as a symbol of luck and prosperity. Genus *Paulownia*, initially classified in the Scrophulariaceae family, has had its genetic affiliation updated based on modern genetic research and now belongs to the *Paulowniaceae* family (Abdel-Moniem et al., 2021; Criscuoli et al., 2022; Hamad, 2023; Icka et al., 2016; Jakubowski M., 2022, Simion, 2009). It comprises numerous species of deciduous trees originating from China and Southeast Asia, but their rapid growth rate and good adaptability to various edaphic-climatic conditions have enabled their spread to Europe, North and Central America, Australia, sometimes becoming invasive (Criscuoli et al., 2022; Guo et al., 2023; Rodríguez-Seoanea et al., 2020; Sławińska et al., 2023; Yadav et al., 2013).

Paulownias are cultivated as ornamental trees but have numerous other economic uses, especially due to the quality of their wood. The wood of many *Paulownia* species and hybrids is soft, low-density, knot-free, dries quickly, does not crack, and is easy to shape, making it highly valued for furniture, sculptures, musical instruments, crafts, boxes, lightweight skis, etc. Although light, it is a hard wood, resistant to water and decay, has dimensional stability, low thermal conductivity, and a high ignition point, recommending it for construction, quality lumber, reinforcement filler for thermoplastic composites, specialized applications requiring fire protection, sandwich structures applied to ships, aircraft, automobiles. Thin stems and branches are used for the production of particle boards, cellulose, paper, biofuels, biomass, etc. (Abdel-Moniem et al., 2021; Baier et al., 2021; Barbu et al., 2022; Costea et al., 2021; Jakubowski, 2022; Langowski et al., 2019; Ptach et al., 2017; Rodríguez-Seoanea et al., 2020).

Other parts of *Paulownia* plants find uses in various fields. Flowers, fruits, leaves, bark are used in traditional medicine in Asian countries, as well as in the pharmaceutical industry, for their antioxidant, anti-inflammatory, antibacterial, antiviral, hypoglycemic, neuroprotective properties, etc. (Costea et al., 2021; Criscuoli et

al., 2022; Guo et al., 2023; Rodríguez-Seoanea et al., 2020). Flower extracts are used in the perfume and cosmetics industry. Flowers and leaves, rich in lipids, carbohydrates, and proteins, are used in human nutrition and animal feed, and the essential oil from flowers, due to its chitosan content, presents great potential for food preservation.

Spreading the use of this species will bring major benefits to the environment, in terms of health and economic aspects of life (Zlati et al., 2024). Flowers also have honey value (Costea et al., 2021; Guo et al., 2023; Hamad, 2023; Langowski et al., 2019).

Due to their ability to grow in different types of soil and climate (Danciu et al., 2016.,), *Paulownia* species, hybrids, and clones from agro-forestry plantations reduce the effects of flooding and soil erosion, help recover abandoned agricultural land, improve soil fertility through nitrogen input, and contribute to the phytoremediation of soils polluted with heavy metals due to their ability to accumulate Cu, Zn, Ca, K, Mg, Na, Fe, Cd (Abdel-Moniem et al., 2021; Barbu et al., 2022; Costea et al., 2021; Fan et al., 2017; García-Morote et al., 2014; Rodríguez-Seoanea et al., 2020).

Compared to other tree species, *Paulownia* has a considerable growth rate, making it an excellent choice for farmers seeking quick profits. In the first 12 months, a *Paulownia* specimen can reach up to 3 meters in height, and in just 3-6 years, it can reach commercial maturity (Wang & Shogren, 1992). Other studies mention growths of 10-20 m in height and 30-40 cm in diameter (DBH), with a volume of approx. 1 m^3/tree at the age of 5–7 years (Hamad, 2023).

For wood production, hybrids obtained from popular species (*P. elongata, P. fortunei, P. tomentosa*) are frequently used, with high productivity and adaptability to the environment (Jakubowski, 2022).

Although they have reduced sensitivity to soil type, they prefer permeable, well-drained soils with a pH between 5 and 8 (optimal 6-7), well supplied with water and nutrients (García-Morote et al., 2014; Szabó et al., 2022). A soil analysis before planting is vital to determine what type of fertilization is necessary, and the application of organic fertilizers can significantly influence plant growth. Also, constant soil moisture, especially in the early stages of development, is very important, as lack of water can lead to slow development and, in extreme cases, even plant death (Aspinall, 1980).

Considering that interest in *Paulownia* agroforestry crops has increased in Europe in recent years (Barbu et al., 2022), they can represent an interesting opportunity for farmers and investors in Romania. However, it is important to conduct further research and consult experts before initiating such a crop, as its success depends on several factors, including proper crop management and identifying potential markets for the products obtained (Stringer et al., 1992).

This paper aims to analyze the effect of irrigation on the growth of plants from two *Paulownia* hybrids ('SuperHybrid Z 07' and 'Cotevisa 2') cultivated in a plantation located in the N-E area of Romania. Both varieties taken into cultivation are appreciated for commercial uses due to their rapid growth and superior wood quality. 'Cotevisa 2' stands out for its accelerated growth rate, reaching commercial dimensions in 3-4 years, making it ideal for wood production. 'SuperHybrid Z07', although growing more slowly, reaches maturity of harvesting in approximately six years. Both varieties are adapted to various climatic and soil conditions, preferring sunny locations and well-drained soils.

MATERIALS AND METHODS

The study was conducted between 2022 - 2024 in a 4-year-old *Paulownia* plantation, part of the "*Paulownia* Șipote" company, located in Șipote commune (55 km from Iași municipality), aimed at producing trees for timber. The biological material consisted of two *Paulownia* biotypes ('SuperHybrid Z 07' and 'Cotevisa 2'), promoted for forest and agricultural plantations due to characteristics that make them attractive to farmers or investors in horticulture and forestry.

'SuperHybrid Z07' (Pao Tong) resulted from the hybridization of three *Paulownia* species (*P. tomentosa x P. fortunei x P. kawakamii*). The material used for establishing experimental plantations was obtained by cloning seedlings from their own nurseries, being part of the elite segment. It requires six years of vegetation to

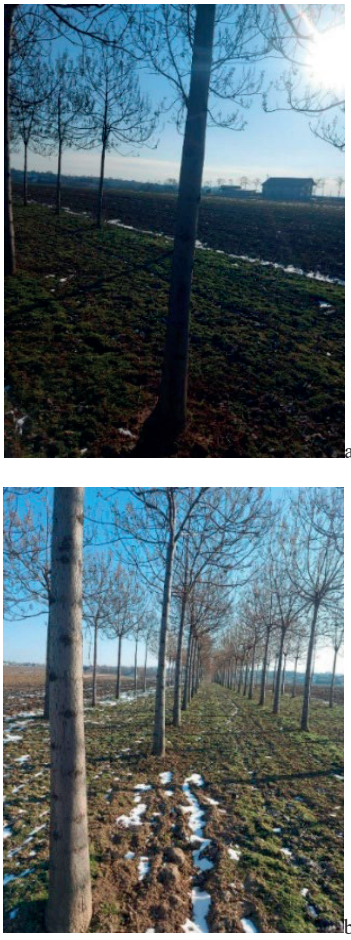
reach minimum economic efficiency and withstands temperatures down to -33°C.
'Cotevisa 2' is a sterile hybrid between *P. elongata x P. fortunei*, which reaches economic efficiency in just four years of vegetation and withstands temperatures down to -25°C.
Experimental crops were established in 2021 with cuttings from *Paulownia* Șipote (Poșta et al., 2022). Planting was done at distances of 4 m between rows and 4 m between plants in the row.
The quantity and quality of *Paulownia* wood production are correlated with the application of appropriate cultivation technology both at establishment and subsequent maintenance of crops, which should meet plant requirements but be adapted to local pedo-climatic conditions. Before planting, the land was cleared (Figure 1.a), deep soil mobilization was done, and organic and chemical fertilizers were administered. Seedlings were planted in 15x15x15 cm holes, and a drip irrigation system was installed for irigated variants. Water was administered 2-3 times a week, 12 liters per watering, depending on weather conditions, especially in critical periods after planting. In the first year, seedlings were protected from herbivorous animals by temporary fencing. Phytosanitary treatments and weed control measures (chemical and mechanical) were also applied.
An essential task for managing and caring for *Paulownia* was technical pruning, aimed at shaping and controlling tree growth. The task was performed after the first year of vegetation (during the dormant period) and consisted of cutting stems at 5 cm above ground level (Figure 1.b). From the shoots emerging in spring, the most vigorous one was chosen to project the final tree stem.



a) b)

Figure 1. Works applied in the experimental plot:
a) preparing the land for planting; b) technical pruning

Experiments were bifactorial and organized in randomized blocks with three repetitions (five plants/repetition). The first experimental factor (biotype) had two variables represented by the cultivated *Paulownia* hybrids: 'SuperHybrid Z 07' and 'Cotevisa 2'. The second factor was irrigation, with two variables: non-irrigated and irrigated (Al-Jurdi et al., 2009).
During the period 2022-2024, the following determinations were made:
- growth increment in height and diameter;
- correlation between analyzed parameters (diameter, height, and volume);
- linear regressions between analyzed parameters (diameter, height, and volume).
From the combination of these factors, four experimental variants resulted:
-V1 - 'SuperHybrid Z07' non-irrigated;
-V2 - 'SuperHybrid Z07' irrigated (Figure 2.a);
-V3 - 'Cotevisa 2' non-irrigated;
-V4 - 'Cotevisa 2' irrigated (Figure 2.b).



a)

b)

Figure 2. Aspects from the irrigated experimental crops:
a) 'SuperHybridZ07'(V2); b) 'Cotevisa 2' (V4)

Biometric determinations conducted during the period 2022-2024 focused on stem height, trunk diameter at 1 m height (diameter at breast height - DBH), the degree of stem branching, and the volume of wood mass of the tree trunks. The method for calculating the volume was adapted from dendrometric tables established for forestry species (Popescu-Zeletin et al., 1957) and from simulations estimating the trunk volume of *Paulownia* trees (Szabó et al., 2022). Based on these data, the growth rate in height, diameter (DBH), and trunk volume was evaluated through comparisons between 2022 and 2024, and linear correlations and regressions were calculated between the analyzed parameters in 2024 (diameter, height, and volume).

For the statistical analysis of the data (Tables 1 and 2), confidence intervals for the mean values, correlation coefficients(r),determination coefficients (R^2) between measured parameters (Apostol et al., 2024; Cojocariu et al., 2024; Ozarchevici et al., 2023), and linear regression lines between parameters (Ungureanu et al., 2023; Chiruță et al., 2023; Draghia et al., 2025) were used. The analysis was performed using the MS Excel application from the MS Office Professional 2016 package, with a significance level of 0.05.

Table 1. Trunk diameter - DBH (cm)

Variants	Years	Average	Confidence Interval*
V_1	2022	4.20	[4.053, 4.347]
	2024	12.40	[12.27, 12.53]
V_2	2022	8.00	[7.869, 8.131]
	2024	18.40	[18.27, 18.53]
V_3	2022	5.30	[5.174, 5.426]
	2024	15.00	[14.10, 15.90]
V_4	2022	10.60	[10.461, 10.739]
	2024	26.80	[26.69, 26.91]

*level of confidence 0.05

Table 2. Total stem height (cm)

Variants	Years	Average	Confidence Interval*
V_1	2022	244.00	[242.418, 245.582]
	2024	410.00	[408.62, 411.38]
V_2	2022	305.00	[303.832, 306.168]
	2024	618.30	[618.19, 618.81]
V_3	2022	204.00	[202.383, 205.617]
	2024	553.00	[551.96, 554.04]
V_4	2022	503.00	[501.988, 504.012]
	2024	896.67	[896.54, 896.80]

*level of confidence 0.05

RESULTS AND DISCUSSIONS

The results regarding the average of the parameters recorded during the mentioned period (total stem height, trunk diameter - DBH, number of branches per plant, volume of wood mass) were documented in Table 3. Stem branching began in all experimental variants in 2024, the third year after establishment, hence the recording of this parameter was only done in 2024. Additionally, trunk volume was evaluated only in 2024, as the results from 2022 were irrelevant due to the small diameter values (Table 3).

It was observed that both in 2022 and 2024, the highest values for trunk diameter and stem height were recorded in the irrigated variants of both varieties (V2, V4).

Regarding the branching capacity of the trees, the non-irrigated variant of the 'SuperHybrid Z07' hybrid shows a much more evident influence of irrigation, in the sense that in the non-irrigated variant (V1), the plants did not form branches, compared to the irrigated variant which formed 4 branches per plant.

Table 3. Averages of analyzed parameters

Variants	2022			2024			
	DBH (cm)	H (cm)	V (m^3)	DBH (cm)	H (cm)	V (m^3)	NR
V_1	4.2	244	-	12.4	410	0.05	0
V_2	8.0	305	-	18.4	618.3	0.13	4
V_3	5.3	204	-	15	553	0.08	2.7
V_4	10.6	503	-	26.8	896.7	0.4	6

Note: DBH - diameter at breast height; H - height total; V - volume; NR - no. of branches

The data from Table 3 served as the basis for calculating the growth increase of *Paulownia*, an indicator resulting from the comparison of the two production years and very important from an economic perspective.

Growth increase in height and diameter (Differences between 2024 and 2022)

The growth increment represents an important analysis for studying the economic evolution of *Paulownia* trees. Regarding the growth increment of DBH between 2024 and 2022 (Table 4), average values varied from 8.2 cm for 'SuperHybrid Z07' non-irrigated (V1) to

16.2 cm for 'Cotevisa 2' irrigated (V4). From the graphical representation of averages, minimums, and maximums of the DBH growth increment (Figure 3), it is observed that the differences between maximums and minimums were relatively small (indicating uniformity of this characteristic). The exception was variant V3, where the amplitudes between values were larger, indicating the reaction of the 'Cotevisa 2' hybrid under non-irrigated conditions through an increase in the non-uniformity of this characteristic.

Table 4. Diameter growth increase (cm)

	V_1	V_2	V_3	V_4
Minimum	7.700	10.000	7.800	15.900
Maximum	8.600	11.000	11.800	16.400
Average	8.200	10.400	9.700	16.200

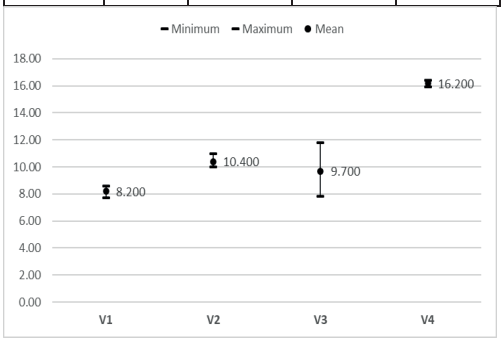


Figure 3. Diameter growth increase (cm)

The height growth increase resulting from the comparison of data from 2024 with those from 2022 highlighted the evolution of the trees in the same order as the DBH increment. More significant height increases were observed in the irrigated variants (V2 and V4), and among the hybrids, the largest differences, both in irrigated and non-irrigated conditions, were found in 'Cotevisa 2' (Table 5).

Table 5. Height growth increase (cm)

	V_1	**V_2**	**V_3**	**V_4**
Minimum	165.000	310.600	348.000	392.000
Maximum	167.000	315.100	350.000	396.300
Average	166.000	313.300	349.000	393.670

The uniformity of this characteristic within each variant can be observed in Figure 4, where for all variants, the maximums and minimums are very close to the average.

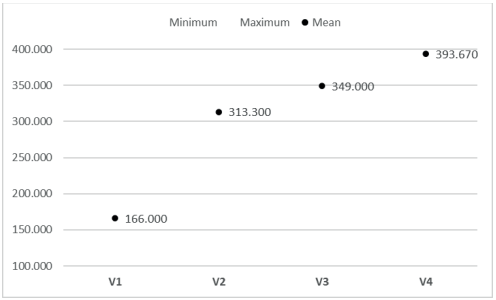


Figure 4. Height growth increase (cm)

Correlations between analyzed parameters (diameter, height, and volume)

Correlation coefficients were calculated for the pairs of characteristics established as variables (Table 6), based on the values recorded in 2024. Regarding the pair of characteristics diameter (DBH) and total height (Table 6), the value of the coefficient r was negative for 'SuperHybrid Z07', both in the non-irrigated variant (V1) and the irrigated variant (V2), which suggests that a greater increase in diameter correlates with a decrease in height growth. However, this correlation is significant only in the non-irrigated variant (r = -0.463). For 'Cotevisa 2', the correlation between DBH/height is positive (as the diameter increases, the height also increases), but significant only in the irrigated variant (V4), where the correlation coefficient was (r = 0.429).

For the pair of characteristics DBH/volume (Table 6), the correlations were negative for the variants of 'SuperHybrid Z07' (V1 and V2) and the non-irrigated variant of 'Cotevisa 2' (V3), but significant only for plants in the non-irrigated variants (V1 and V3).

It can be concluded that the volume of wood material is directly and significantly influenced by the tree diameter only in the irrigated variant of 'Cotevisa 2' (V4). A particular situation was recorded in the relationship between height and volume (Table 6).

Table 6. Correlation between analyzed parameters (2024)

V_1	Height	Volum
Diameter	-0.463	-0.529
Height	***	0.802
V_2	Height	Volum
Diameter	-0.157	-250

Height	***	0.898
V_3	Height	Volum
Diameter	0.144	-0.053
Height	***	0.867
V_4	Height	Volum
Diameter	0.429	0.505
Height	***	0.779

In all variants, the correlation was direct and strong, with r values ranging between 0.779 and 0.898, leading to the conclusion that intense growth in height results in a significant increase in wood mass volume.

Linear regressions between analyzed parameters

The relationship between variables was evaluated by calculating the coefficient of determination (R^2), which represents the proportion of the total variation of the dependent variable that can be explained by the independent variable. As with correlations, the data used for calculating linear regressions were those corresponding to the year 2024.

Initially, a multilinear regression was established between the dependent parameter (wood mass volume) and the independent parameters (tree height and diameter - DBH). From the result, it is observed from the p-value (p-value = 0.4354 > 0.05 significance level) that the tree diameter parameter is not significant (Table 7).

Table 7. Multilinear regression between wood mass volume based on height and diameter, data for 2024 (V1)

Regression Statistics				
Multiple R	0.821378901			
R Square	**0.6746633**			
Adjusted R Square	0.581709957			
Standard Error	0.007468066			
Observations	10			
	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-1.3590	0.6327	-2.1478	**0.0688**
Diametrul	-0.0116	0.0140	-0.8273	**0.4354**
Înălțimea	0.0038	0.0013	2.9137	**0.0225**

The decision was made to analyze the linear regression between wood mass volume and tree height across all variants (Figures 5, 6, 7, and 8). To construct the regression lines, the dependent variable was considered to be the wood mass volume of the tree, and the independent variable was the total height of the tree. This allows for the anticipation of volume (m^3) based on the value of the other analyzed variable.

In Figure 5, the regression line between volume and height is presented for variant V1 ('SuperHybrid Z07' non-irrigated). The R^2 value indicates that for this variant, 64% of the variation in volume, as a dependent variable, is due to the relationship with total height (as an independent variable).

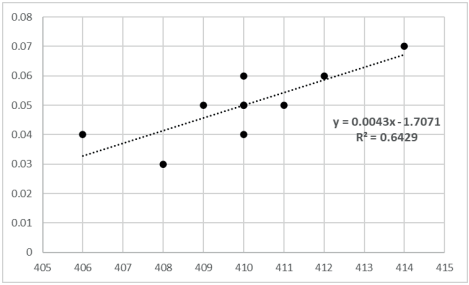


Figure 5. Linear regression line for 'SuperHybrid Z07' non-irrigated variant (V1)

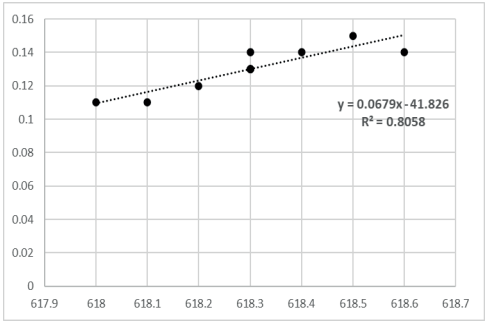


Figure 6. Linear regression line for 'SuperHybrid Z07' irrigated variant (V2)

Under irrigation conditions for the 'SuperHybrid Z07' hybrid (V2), the dependency of volume on stem height is significantly higher (80%), with $R^2 = 0.8058$ (Figure 6).

The coefficient of determination calculated for 'Cotevisa 2' indicated that the variation in volume is due to height by 75% under non-irrigated conditions (Figure 7) and only 60% under irrigation conditions (Figure 8).

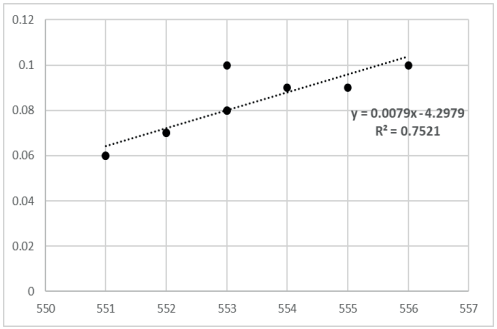


Figure 7. Linear regression line for 'Cotevisa 2' non-irrigatedvariant (V3)

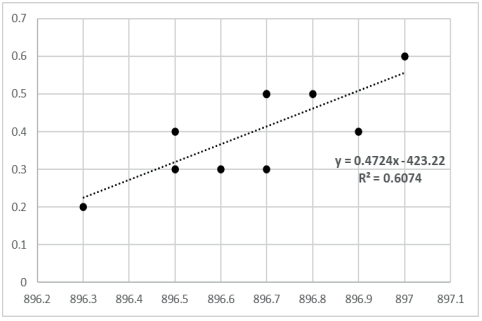


Figure 8. Linear regression line for 'Cotevisa 2' Irrigated variant (V4)

CONCLUSIONS

The research conducted led to the following conclusions:

- the quantity and quality of *Paulownia* wood production are correlated with the application of appropriate cultivation technology both at the establishment and during the subsequent maintenance of the crops.
- the increase in DBH between 2024 and 2022 recorded average values ranging from 8.2 cm for the non-irrigated 'SuperHybrid Z07' (V1) to 16.2 cm for the irrigated 'Cotevisa 2' (V4).
- the correlation between height and volume, indicating that in all variants, the correlation was direct and strong, with r values ranging from 0.779 to 0.898, suggesting that intense height growth leads to a significant increase in wood mass volume.
- under irrigation conditions for the 'Super Hybrid Z07' hybrid (V2), the dependence of volume on stem height is much greater (0.80%).
-the coefficient of determination calculated for 'Cotevisa 2' indicated that the variation in volume is due to height by 75% under non-irrigated conditions and only by 60% under irrigated conditions.
-linear regression between wood mass volume and tree height was conducted to accurately assess progress over the two years of analysis, determining a significant determination coefficient (between 0.6074 and 0.8058).

So, both varieties have great prospects to be used on a large scale.

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REVIEW ON THE CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *LAVANDULA* SPP. AND ITS APPLICATIONS

Gabriela OPREA (BUTNARIU), Monica Luminița BADEA, Adina NICHITA, Doru Ioan MARIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: gabriela3020022002@yahoo.com

Abstract

Lavandula spp., known as lavender, belongs to the Lamiaceae family and is an aromatic plant with various uses, the most important of which are pharmaceutical (medicinal plant), food, cosmetics, detergents, industrial, perfumery, etc. The Lamiaceae family includes 47 species of flowering plants, and among Lavandula spp. we can mention: Lavandula angustifolia, Lavandula dentata, Lavandula lanata, Lavandula latifolia, Lavandula multifida, Lavandula pedunculata, Lavandula stoechas, Lavandula viridis, etc. This paper reviews studies and research conducted in the specialized literature over the last 10 years (2014-2024) on the characteristics and properties of Lavandula spp., thus providing a comprehensive overview of the use of essential oils and extracts in various fields. The variability of essential oil is discussed according to the species of lavender, geographical area (where it is cultivated), plant material (flowers, leaves, whole plant), and extraction methods.

Key words: *chemical compounds,essential oil, Lavandula, multiple applications.*

INTRODUCTION

Lavender *(Lavandula angustifolia* Mill.), also known as English lavender (syn. *Lavandula officinalis* L. Chaix et Vill., *Lavandula vera* DC), belongs to the *Lamiaceae* family.

The *Lamiaceae* family includes 47 species of plants (with flowers), and the genus *Lavandula* spp. comprises 39 species and numerous hybrids and varieties, which grow on all continents (North Africa, Southwest Asia, North and South America, Europe, India, and the Arabian Peninsula (Lis-Balchin, 2012; Messaoud, 2012).

The genus *Lavandula* spp. includes the following species: *Lavandula angustifolia* (English lavender), *Lavandula x allardii*, *Lavandula coronopifolia*, *Lavandula dentata* (toothed lavender/fringed lavender), *Lavandula x intermedia* (lavandin), *Lavandula lanata* (woolly lavender), *Lavandula latifolia* (Portuguese lavender), *Lavandula luisieri*, *Lavandula multifida* (Egyptian lavender), *Lavandula officinalis* (medicinal lavender), *Lavandula stoechas* (Spanish lavender), *Lavandula viridis* (Green Lavender) (https://mymediterraneangarden.com/types-of-lavender/,https://www.pinterest.com/pin/140806232692625/).

Lavandula spp. is a medicinal plant with a specific aroma, used in traditional medicine for many years, but also in other industries (*detergents, perfumery, pharmaceuticals*), and in landscaping as an ornamental plant in parks and gardens, as it is a plant that improves the soil in which it is grown (Chrysargyris et al., 2016; Giuliani et al., 2023; Laza and Rácz, 1975; Muntean et al., 2007; Patil et al., 2022*).*

The main objective of this study is to provide a systematic overview of the essential oil derived from species of the genus *Lavandula*, to highlight the chemical composition of the volatile oil, as well as the diversity of its applications.

According to Giuliani (2023), *Lavandula angustifolia* Mill. has high commercial value due to its trichomes (secretory hairs), which are responsible for producing volatile substances.

Essential oils derived from *Lavandula angustifolia* Mill. are rich in linalyl acetate, which gives them their characteristic scent, coumarin, tannin, a bitter principle, and the essential oil obtained by distilling the inflorescences (flowers) can be used in human

medicine (effective in treating early and moderate depression) (Shafiee-Kandjani et al., 2023), in the pharmaceutical industry (obtaining pharmaceutical products based on essential oil), cosmetics (creams, soaps), and in the perfume industry (cologne, perfumes) (Constantinescu et al., 2004).

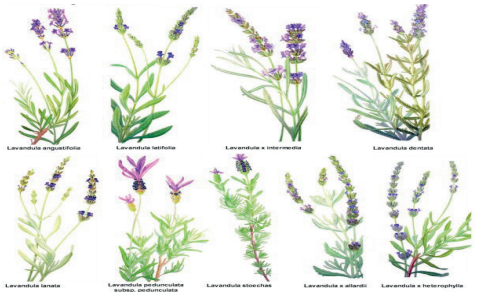


Figure 1. Various cultivars of the genus *Lavandula* spp.
Source: https://in.pinterest.com/pin/12596073951347604/

Lavandula angustifolia Mill. is a highly prized species worldwide, which means that lavender is cultivated (on all continents) and researched (all over the world), due to the species' multiple uses in various industries such as: the food industry, the paint industry, the detergent industry, the pharmaceutical industry, in medicine, but also as an ornamental (decorative) plant.

It is very important to note that if we want to cultivate lavender (especially when cultivating a specific variety), it is essential to know how to differentiate between: the use of the species for the production of essential oil, the use of the species for the production of cut flowers, or the use of the species strictly for ornamental purposes. At the same time, lavender has multiple uses, including: antibacterial, antiseptic, antispasmodic, aromatic, carminative, cosmetic, culinary, decorative, expectorant, medicinal, and stimulant.

Worldwide, research conducted on the *Lavandula* spp. species has extensively described both the chemical composition of the essential oil obtained by various methods and its main components (essential oil).

The chemical composition of the essential oil differs depending on the area of cultivation (continent, country), the species cultivated, and the variety, so that significant differences in chemical compounds have been reported. Linalool (the most important chemical compound) has shown different concentration values depending on the results obtained by different researchers (Table 1).

Table 1. Linalool concentration in different countries where research on lavender essential oil has been conducted

Country	Linalool (%)	Sources/Year
Lavandula angustifolia Mill.		
Algeria	22,30	Djenane și colab., 2012
Bulgaria	30.10-33.70	Ognianov, 1994
Bulgaria	23.13-35.52	Todorova și colab., 2023
China	24.30	Xiaotiana și colab., 2020
Croaia	3.97	Blazekovic și colab., 2018
Cyprus	0.17-0.24	Chrysargyris și colab., 2016
France	9.30-68.80	Beale și colab., 2017
France	9.30-68.80	Lawerence, 1993
Greece	20.18	Adam și colab., 1998
Hungary	5.1-62.70	Detar și colab, 2021
India	23.60	Raina și Negi, 2012
India	35.30	Fakhari și colab., 2006
Italy	32.70	Evandri și colab., 2005
Italy	39.31	Alteriis și colab., 2022
Poland	15.10-21.77	Walasek-Janusz, 2022
Romania	18.46-39.50	Oroian și colab., 2019
Romania	24.15-50.84	Goncearіuc și colab., 2018
Serbia	28.0-37.0	Lakusik și kolab., 2014
Syria	27.30-34.70	Al-Wassouf și colab., 2018
U.S.A.	29.00-33.00	Wang și colab., 2021
Lavandula intermedia		
Croatia	57.10	Blazekovic și colab., 2018
Italy	41.60	Garzoli și colab., 2019
Poland	25.53-29.56	Walaseck-Janusz, 2022
Romania	21.90	Marincaș și Feher, 2018
Lavandula latifolia		
India	9.1	Al-Ansari și colab., 2021
Spain	30.34	Mendez-Tovar și col., 2016
Lavandula officinalis		
Argentine	53.50	Martuccci și colab., 2015
Spain	34.34	Marin și colab., 2016
Lavandula viridis		
Porugal	0.93	
Lavandula stoechas		
Marocco	0.7	En-Zoubi și colab., 2022

Thus, among the main components present in lavender essential oil, we can mention monoterpenoids (most importantly linalool, linalyl acetate, 1,8-cineole, beta-ocimene, terpine-4-ol, and camphor, another important product).

Lavender essential oil also contains sesquiterpenoids (beta-caryophyllene) and other terpenoid compounds (Bikmoradi et al., 2017).

The importance of essential oil (volatile)

Research has shown that essential oil obtained from species of the genus *Lavandula* spp. (Table 2) has a very high content and many beneficial effects on the human body, and is also used in various fields (Plotto and Roberts, 2001; Cavanach et al., 2002, Hyldgaard et al., 2012; Gutierez, 2008; Adaszynska et al., 2013; Nieto, 2017; Sarkik and Stappen, 2018), including:

Cosmetics and pharmaceuticals - the perfume industry (cologne) and cosmetics (body care, detergents, face creams, hand creams, skin care, shampoos, shower gels, soaps,), in pharmaceuticals (flavoring ointments, infusions, tinctures - *Lavandula angustifolia* being preferred due to its strong scent), industrial preparations with sedative and cholagogue effects and for internal use, pharmaceutical preparations such as *Acetum Aromaticum* (aromatic vinegar), Spiritus Lavandulae (lavender-flavored alcohol), and Tinctura vulneraria (wound tincture). (Luchian et al., 2017, Păun et al., 1988, Pârvu et al., 2006, Prisăcaru et al., 2009)

Dendrological and ornamental - real interest from an ornamental point of view (flower color - from red to dark blue, fragrance, foliage persistence), dwarf species suitable for parks and gardens (simple borders or in combination with other species, in rockeries alongside other perennial species), cultivated alone or in various floral compositions. (Iliescu, 2005, Pârvu, 2006)

Food - preparation of cakes, dishes, juices, honey (very good nectar-producing capacity - nectar- and pollen-producing species), yielding about 50-100 kg of high-quality honey/ha, with a pleasant taste and specific aroma, medium economic and beekeeping value (Pârvu, 2006).

Industry - in the porcelain industry (Yegorova, 2011), in ceramics (good solvent and paint fixative), insect repellent (for combating moths), tobacco (anti-asthmatic cigarettes). (Constantinescu et al., 2004, Munteanu, 1988 cited by Roman, 2008, Muntean, 1990, Stănescu et al., 2014, Pârvu, 2016).

Medical and therapeutic in both human and folk medicine (internal use - antiseptic, antispasmodic, healing, and soothing action, and external use - when we are tired, for rheumatism, whooping cough, flu, bronchitis, digestive disorders, and headaches). It increases bile secretion, helping to relieve stomach pain, as well as in traditional veterinary medicine (internal use - to treat lack of thirst, a few drops of essential oil are added to water, encouraging animals to drink (Bakkali et al., 2008; Hassiotis et al., 2010; Ion et al., 2008, Păun et al., 1988, Pârvu, 2006, Pârvu et al., 2016).

Lavender essential oil, obtained from plants of the *Lavandula* genus, has various beneficial effects on the body due to the active compounds in its composition.

Other therapeutic uses of the essential (volatile) oil

Antifungal action: Lavender essential oil has antifungal properties and can be used to treat fungal infections, such as athlete's foot, or to inhibit the fungus Ustilaginoidea virens (inhibition of hyphal growth, dry weight of mycelium, conidia germination, and conidia production) that attacks rice crops (Fu et al., 2024).

Antibacterial action: Lavender essential oil has antibacterial properties and can be used to treat bacterial infections such as acne, as well as promising antibacterial activity against some strains of *S. aureus* and *E. coli* (Soulaimani et al., 2025).

Anti-inflammatory action: Lavender essential oil has anti-inflammatory properties and can be used to relieve inflammation and reduce (skin) inflammation in atopic dermatitis (Duan et al., 2024).

Sedative action: Lavender essential oil has sedative properties and can be used to help reduce stress and anxiety, especially in children who need to undergo endoscopy (Aydin et al., 2025).

Analgesic action: Lavender essential oil has analgesic properties and can be used to help relieve headaches and other pains, as well as to treat chronic prostatitis with the help of creams based on lavender essential oil (Wang et al., 2025).

Antispasmodic action: Lavender essential oil has antispasmodic properties and can be used to help relieve muscle spasms or prevent pain in pediatric patients (children) undergoing surgery (tonsillectomy), reducing postoperative pain (Ahmadi et al., 2023).

***Antidepressant action**:* Lavender essential oil can be used to help alleviate symptoms of depression and other mental health issues through the effects of aromatherapy. Inhaling the essential oil has a calming effect, reducing depression and leading to more restful sleep in patients who have suffered strokes (Yin et al., 2024).

Due to these properties, lavender essential oil is frequently used as an ingredient in medicinal and cosmetic products.

Table 2. The use of essential oil derived from lavender species of the genus *Lavandula* spp.

Species	Use
1. Lavandula x allardii	cosmetics, food industry, perfume industry, pharmaceutical industry
2. Lavandula angustifolia Mill.	acaricidal effect, allelopathy, aromatherapy, cosmetics, food industry (food products), insecticidal effect, soil modification, repellent, therapeutic potential, veterinary products
3. Lavandula coronopifolia	food industry (food products), therapeutic potential, *soil* phytoremediation, veterinary use (veterinary products)
4. Lavandula dentata	therapeutic potential
5. Lavandula x intermedia	Food industry (in nutrition), in medicine (anesthetic)
6. Lavandula latifolia	food industry, medicine (medicinal purpose), use as insecticide
7. Lavandula luisieri	in medicine (antifugal drugs) veterinary medicine (for appetite and thirst in animals), use as insecticide
8. Lavandula multifida	therapeutic potential
9. Lavandula officinalis	medicine (medicinal purpose)
10. Lavandula stoechas L.	cosmetics, perfume industry, pharmaceutical industry, therapeutic potential,

The species of lavender and the uses of the essential oil extracted from each species will be listed below as follows:

1. ***Lavandula x allardii*** *- cosmetics, food industry, perfume industry, pharmaceutical industry* (Chasiotis et al., 2001);

2. ***Lavandula angustifolia*** **Mill.** *- acaricidal effect* (Perrucci et al., 1996; Kaya, 2010), *allelopathy* (Sidorenko et al., 1995), *aromatherapy* (Lis-Balchin & Hart, 1999; Evandri et al., 2005; Donatello et al., 2020), *cosmetics* (Koniger, 1997; Fakhari et al., 2005; Kunicka-Styczynska, 2009; Adaszynska et al., 2013; Kunicka-Styczynska et al., 2015; Saeed et al., 2023), *food industry* (*food products*) (Fenaroli, 1998; Fakhari et al., 2005; Adaszynska et al., 2013; Fascella et al., 2020), *insecticidal effect* (Carson & Riley, 1995; Sertkaya et al., 2010; Khosravi et al., 2013; Yazdani et al., 2013; Julio et al., 2014; El Abdali et al., 2022; Ez-zoubi et al., 2022), *soil modification* (Yohalem et Passey, 2011), *repellent* (Warren et al., 1997) *therapeutic potential* (Bertram, 1995; Buyukokuroglu et al., 2003; Hajhashemi et al., 2003; Woronuk et al., 2011; Raut & Karuppayil, 2014; Koziol et al., 2015; Kivrak, 2018; Malcolm & Tallian, 2018; Bialon et al., 2019; Donatello et al., 2020; Zeinab et al., 2020; Detar et al., 2021; Doha et al., 2021; Firoozeei et al., 2021; Villalpando et al., 2022; Saeed et al., 2023), *veterinary products* (Wren, 1988; Ercan & Esma, 2019);

3. ***Lavandula coronopifolia*** *- food industry* (*food products*) (Preedy, 2016), *therapeutic potential* (Said et al., 2015; Hasanin et al., 2020; Naseef et al., 2022.), *soil phytoremediation* (Shafagha et al., 2012), *veterinary use* (*veterinary products*) (Ferguson,et al, 2013.);

4. ***Lavandula dentata*** *- therapeutic potential* (El Abdali,2022; Bouyahya et al., 2023);

5. ***Lavandula x intermedia*** *- food industry* (*in nutrition*) (Fenaroli, 1998), *in medicine* (*anesthetic*) (Krasteva et al., 2021, Yigit et al., 2022);

6. ***Lavandula latifolia*** *- food industry* (Fenaroli, 1998; Mendez-Tovar et al., 2016), *medicine* (*medicinal purpose*) (Rodrigues et al., 2012; Herraiz-Peñalver et al., 2013; Gayoso et al., 2018; Al-Ansari et al., 2021), *use as insecticide* (Al-Ansari et al., 2021);

7. ***Lavandula luisieri*** *- in medicine* (*antifugal drugs*) (Zuzarte, et al., 2012), *disinfectant solution* (*antiseptic*) (Gonzalez-Coloma et al., 2011);

8. ***Lavandula multifida*** *- therapeutic potential* (Benbelaid, 2012);

9. ***Lavandula officinalis*** *- medicine* (*medicinal purpose*) (Meftahizade et al., 2011; Imene, 2012; Et-Touys et al., 2016; Kivrak, 2018).

10. ***Lavandula stoechas*** **L**. *- cosmetics* (Bouyahya et al., 2017), *perfume industry* (Repici, 2019), *pharmaceutical industry* (Repici, 2019), *therapeutic potential* (Bouyahya et al., 2017; Chograni et al., 2021; Rasheed et al., 2023).

Harvesting

Species of the genus *Lavandula* spp. shall be harvested at the optimum time, which shall be determined with precision. Thus, the flowering period of the species *Lavandula angustifolia* Mill. (Lavender) comprises four distinct stages (phases): *budding* (inflorescences are budding); *the beginning of flowering* (25% of the flowers are in bloom); *full flowering* (50% of the flowers are in bloom); *the end of flowering* (85% of the flowers are past their prime), where harvesting must be carried out before 12 noon (because the oil content increases continuously), and after 4 p.m. (it decreases significantly), noting that the most recommended hours for harvesting are 10 a.m. to 12 p.m. (on small areas) and 9 a.m. to 2 p.m. (on large areas), harvesting not being recommended in cloudy or cool weather, this being done manually with a sickle or mechanically with the help of machines specially mounted on tractors.

Chemical composition of essential oil

Currently, in the specialized literature, as well as in research conducted on the genus Lavandula spp., there is a wealth of data on the composition of essential oil (Cavanagah and Wiolkinson, 2005; Bombarda et al., 2008; Gyrai et al., 2008; Baydar and Kineci, 2009, Danh et al., 2013; Binello et al., 2014; Aprotosoaie et al., 2017; Dris et al., 2017; Tardugno et al., 2019; Detar et al., 2020; Giuliani et al., 2023)

All results obtained regarding the composition of essential oil mention that it (the oil) is greatly influenced by the origin of the species, the cultivated genotype (Munoz-Bertomeu et al., 2007; Stanev, 2010),

the stage of development of the species (Lacusik et al., 2014), agronomic factors (plant growth and development) as well as pedoclimatic factors (climate and soil), agrotechnical factors (cultivation technology applied, soil tillage, sowing, crop care, harvesting) (Renaud et al., 2001; Angioni et al., 2006; Pinto et al., 2007; Erbaş & Baydar, 2008, Kara & Baydar, 2013; Camen et al., 2016; Garcia-Caparros et al., 2019; Lyczko et al., 2019; Fascella et al., 2020; Pecanha et al., 2021), plant parts (Geisel et al., 2004; Lakusic et al., 2014) extraction method, storage and processing of biological material (Chemat et al., 2006; Karapandzova et al., 2014; Babu et al., 2016; Duskova et al., 2016; Salata et al., 2020;).

Numerous authors who have studied the chemical composition of essential oil have demonstrated, through their research findings, the presence of significant differences in its composition. These variations are primarily attributed to the extraction method used, such as hydrodistillation or microwave-assisted extraction.

Thus, microwave-assisted extraction significantly accelerates the process of obtaining essential oil, without causing notable changes in its chemical composition.

According to the scientific literature, essential oil contains over one hundred chemical constituents, the main compounds being linalool (with a concentration ranging from 9–69%) and linalyl acetate (1.2–59%) (Beale et al., 2017)

The quality of essential oil derived from *Lavandula* spp. is determined by its high content of linalool and linalyl acetate (Figure 2 and Figure 3), as well as by the ratio in which these two components are present in its chemical composition (Beale et al., 2017).

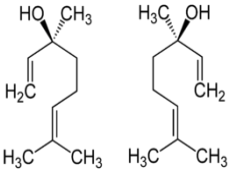


Figure 2. Linalool
Source: https://sk.wikipedia.org/wiki/Linalool

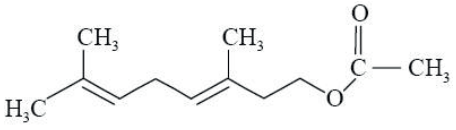


Figure 3. Linalyl acetate
Source: https://alchetron.com/Linalyl-acetate

According to Noyraska (2023), essential oil is actually the product generated from an aromatic plant through the process of distillation (hydrodistillation), which is then followed by the process of separation from the aqueous phase, bearing the generic name of essential oil (EO). Dias (2021) mentions that essential oil represents a complex liquid

mixture of various secondary metabolites (different concentrations), which are classified into two main groups as follows: ***hydrocarbons*** (*diterpenes, monoterpenes, sesquiterpenes*) and ***oxygenated compounds*** (hydrocarbon derivatives), including a*lcohols, aldehydes*, *ketones*, *esters*, *oxides*, etc.

Both the quantity and quality of essential oil obtained from Lavandula spp. are influenced by several factors, including: plant material, age of the plant (bush), the health of the plant (bush), the soil and climate conditions during the harvesting of the plant material, the conditions for the growth and development of the plants (bushes), the method of drying the plant material, and the method used to extract the essential oil, all play a very important role.

Essential oils extracted from medicinal plants, particularly from species belonging to the *Lavandula* spp. genus, have been known and used since ancient times. These oils exhibit a wide range of biologically active properties, including antimicrobial, antioxidant, antitumoral, antiseptic, digestive, antispasmodic, and neuro-sedative effects. Moreover, essential oils have been studied as alternative sources to commercial pesticides for ecological preservation, with their active compounds forming the basis of effective defense strategies against herbivorous pests

Significant differences are observed among the cultivated genotypes. Research indicates that, in some cases, the highest concentration of linalool (%) was recorded in the middle of the flowering period, while the highest concentration of linalyl acetate (%) was observed at the end of flowering. In other cases, research shows that large amounts of linalool were recorded (from flowering to the end of the flowering phenophase), and the concentration of linalyl acetate decreased considerably (Baydar and Kineci, 2009) Cantor et al., 2018; Detar et al., 2011).

In other cases, various researchers mention that the three chemical compounds (linalool, terpinen-4-ol, and 1,8-cineole) were found in very high concentrations when the inflorescences (flowers) were in the bud stage.

It is recommended that inflorescences be harvested in the early part of the day because that is when they contain the highest amount of oil and active ingredients. They are detached from the inflorescence axis, or the entire inflorescence is harvested (Constantinescu, 2004).

The raw material is provided by the inflorescences (Lavandulae flos, Lavanduale angustifoliae flos) (Muntean, 1996).

Lavender flowers (*Lavandula angustifolia* Mill.) contain essential oil (0.7-1.4%) which is composed of linalyl acetate (30-40%), linalool acetate, geraniol, nerol, lavandulol, borneol, (+) citronellol, (+)-terpinen-1-01, (+)-epoxy-dihydro-linalool, isogeraniol, cumin alcohol, traces of α-bisabolol, amyl and isoamyl alcohol, n-hexyl alcohol, valeric, isovaleric, propionic, caproic acids, terpenic hydrocarbons, coumarin, herniarin, furfural, etc., tannin, a bitter principle, mineral substances. The flowers are characterized by a pleasant, strongly aromatic scent and a slightly bitter taste. (Pârvu et al., 2016).

Lavender contains volatile oils (approximately 1.5% dry matter), which contain at least 34% esters, tannins (5-10%), coumarins and furanocoumarins (herniarin), flavonoids (luteolin), phytosterols, sterolic saponosides (rosmarinic, ursolic, and oleanolic acids). (Burzo et al., 2005, Burzo, 2013)

We also find: coumarins (herniarin), anthocyanins (delphinidin, malvidin), flavones (luteolin), phenolic acids (rosmarinic acid, chlorogenic acid), triterpenes (ursolic acid, oleanolic acid, betulinic acid, pomilic acid-3,19-dihydroxy-12-ursen-28-oic acid), betulin (Lis-Balchin, 2002 cited by Burzo I, 2015).

Monoterpenes are found in concentrations of 50-90% in lavender flowers, sesquiterpenes in concentrations of 7.5-15.0%, and triterpenoids have the lowest concentration (Bakkali et al., 2008; Lesage-Meessen et al., 2015; Chrysargyris et al., 2016).

The chemical compounds are many and varied, but they vary depending on the species cultivated. The content of oxygenated monoterpenes differs from species to species (cultivated species), and in the case of *Lavandula angustifolia* Mill. (36-93%), *Lavandula x intermedia* (68-93%), *Lavandula stoechas* (46-93%), and *Lavandula latifolia* (85-94%).

Additionally, oxygenated monoterpenes have been found in high concentrations in the species *Lavandula lusieri*, *Lavandula*

pedunculata, and *Lavandula viridis.* The species *Lavandula angustifolia*, *Lavandula luisieri*, and *Lavandula stoechas* have a high content (more than 20%) of sesquiterpenes in their essential oil (Aprotosoaie et al., 2017).

Among the monoterpenoids of fatty acids found in lavender inflorescences, the most important are: linalool, terpinen-4-ol, α-terpineol, borneol, lavandulol, linalyl acetate, lavandulyl acetate, geranyl acetate, geranyl propionate, camphor, fenchone, thujone, and 1,8-cineole, while the most frequently reported monoterpenes found in lavender fatty acids are: limonene, cis-β-ocimene, and trans-β-ocimene. From the sesquiterpenes group, we can mention: β-caryophyllene, β-farnesene, caryophyllene oxide, and viridiflorol (Chrysargyris et al., 2016).

The volatile oil contains a mixture of mono- and sesquiterpenes, of which borneol (40.73%) and tau-cadinol (27.02%) account for the largest proportion. Other substances identified in lavender essential oil had the following proportions: linalool (4.51%), r-cadinene (3.44%), ß-phellandrene (2.97%), camphor (2.79%), α-pinene (1.96%), α-santalene (1.13%), caryophyllene oxide (1.17%), cubenol (0.94%), neryl acetate (0.81%), p-cymene-8-ol (0.69%), bornyl acetate (0.66%), geranyl acetate (0.56%) and α-terpineol (0.41%). (Burzo and Toma, 2013 cited by Burzo, 2015)

By analyzing the volatile oil extracted by Boelens (1986), 44 components were identified, including linalool (41.70%), 1,8-cineol (26.30%), and camphor (12.80%). Concentrations higher than 1% were found for ß-pinene (2.10%), α-bisabolene (1.90%), α-pinene (1.80%), ß-caryophyllene (1.40%), limonene (1.10%), linalyl acetate (1.10%), and α-terpineol (1.00%). Concentrations lower than 1% were determined for borneol, camphene, lavandulol, sabinene, terpinen-4-ol, δ-cadiene, α-humulene, bornyl acetate, trans-linalool oxide, α-p-dimethylstyrene, isoborneol, cis-linalool oxide, myrcene, (E)-β-ocimene, 3-octanone, 1-octenol-3, α-terpinene, γ-terpinene, terpinolene, coumarin, geraniol, (Z)-beta-ocimene, 1-octanol, α-phellandrene, butyl acetate, caryophyllene oxide, dihydrocoumarin, eugenol, 1-hexanol, hexyl acetate, isoamyl acetate, nerol, and α-thujen. (Burzo et al., 2005; Prisăcaru et al., 2009).

The leaves and stems contain small amounts of essential oil. The organic substances highlighted contain N, P, K, Ca, Mg, B, Fe, Mn, and Cu.

Essential oil production varies with the age of the plant: it reaches its maximum after approximately 5-7 years and becomes very low after 10-12 years. The maximum oil content is found at the beginning of flowering between 9 a.m. and 2 p.m. (Pârvu et al., 2016).

The flowers of *Lavandula angustifolia* Mill. contain essential oil (volatile) in quantities that depend on the variety, species, and time of harvest, as well as linalool acetate, lavandulol, terpenic hydrocarbons, tannins, minerals, etc. Dried lavender flowers contain 1.5-3% essential oil, and fresh flowers contain 0.55-1.5% - linalool (up to 30%), linalyl acetate (up to 40%), geraniol, linalool valerate, borneol, linalyl butyrate. The flowers also contain triterpenic acids, flavonoids, resins, coumarins, bitter principles, cineol, tannins, and minerals (Luchian et al., 2017).

Lavender also has active ingredients such as coumarins and caffeic acid derivatives (Rîșca, 2016).

CONCLUSIONS

The volatile oil of *Lavandula* is highly valued for its complex chemical composition, especially its high content of linalool and linalyl acetate, which confer multiple therapeutic and cosmetic properties. The variability of its active compounds depends on the species, cultivation conditions, and extraction methods, influencing its efficacy across various applications. Due to its antimicrobial, antioxidant, and sedative effects, *Lavandula* oil holds significant potential in pharmaceutical, food, and cosmetic industries. Furthermore, its use as an ecological alternative to synthetic pesticides underscores its importance in sustainable agriculture. Continuous standardization and research are essential to fully harness its properties and ensure the quality of lavender-based products.

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RESULTS OF THE INTRODUCTION OF SPECIES OF THE GENUS *SALIX* L. IN UKRAINE

Liubov ISHCHUK[1], Volodymyr HRABOVYI[1], Halyna ISHCHUK[2]

[1]Sofiyivka National Dendrological Park of the National Academy of Sciences of Ukraine, 12A Kyivska Str., Uman, Ukraine
[2]Uman National University of Horticulture, 1 Instytutska Str., Uman, Ukraine

Corresponding author email: ishchuk29@gmail.com

Abstract

The aim of the study of the study was to analyze the success of the introduction of Salix species in the Right-Bank Forest-Steppe of Ukraine based on the characteristics of their growth and development, winter hardiness and drought tolerance. Ten of the autochthonous species are found in Ukraine on the southern or southeastern border of their ranges, which is explained by climate change from the northwest to the southeast of the country. Two species change their life form from a "tree" to a "bush" under conditions of introduction. Six species have not fully lignified shoots at the end of the growing season, which is an indicator of their lower winter hardiness. In Ukraine, the introduced species of the Salix genus form viable seeds, however, they do not form self-seeding and have a full (10 species) or good (6 species) degree of acclimatization. It has been established that the limiting factors for the expansion of the Salix cultural range in Ukraine are high temperature and low humidity of air and soil in summer, which leads to a decrease in their drought resistance.

Key words: *cultural area, introduction, acclimatization, life form, «tree»-«shrub», winter hardiness, drought tolerance.*

INTRODUCTION

The introduction of plants is the most important means of enriching the cultivated flora and an integral component of measures aimed at increasing plant raw material resources. In Ukraine, species of the genus *Salix* L. are widely used in landscaping, forestry, phytomelioration, phytoenergy, the chemical industry, and phytopharmacology. The natural flora of Ukraine includes 23 autochthonous willow species belonging to 15 sections (Fuchylo-Sbytna, 2009; Ishchuk, 2015b). These species differ in habitus, resistance to abiotic and biotic factors, modes of reproduction, and chorology. However, the broad range of applications of willows in various sectors of the national economy necessitates the search for ways to expand their assortment through introduction and selection. The issue of bioecological challenges in urban green construction is also relevant for various regions of Ukraine, where cultural phytocenoses of forest and park types predominate but are significantly affected by ecological and anthropogenic factors (Boiko et al., 2024). Through the reconstruction of plantations by introducing *Salix* species, it is possible to improve the ecological-cenotic and aesthetic condition of urban plantings (Rogovskyi et al., 2023).

The prediction of the successful introduction of *Salix* L. species, particularly the expansion of their cultivated range, is impossible without analyzing the current geographical distribution of species, the ecological characteristics of their habitats, and the genesis of floras (Kokhno-Kurdyuk, 1994; Yatsyk et al., 2017). Due to climate change, as well as the relatively high resistance of willows to low temperatures and diverse edaphic conditions, many *Salix* species are expanding not only their natural but also their cultivated range.

The global flora of willows comprises 300 *Salix* species (Rehder, 1949; Elias, 1980; Skvortsov, 1999). For North America, Elias (1980) reports 80 species, three of which - *S. alba* L., *S. fragilis* L., and *S. viminalis* L. - are part of Ukraine's natural flora. The *Native Trees of Canada* (1950) handbook describes 250 *Salix* species, of which only 75 have a natural distribution in North America. The American publication *Catalog of Cultivated Woody Plants* (1994) lists 30 willow species for the northeastern United States alone. In the United Kingdom and Ireland, 18 willow species are recorded (Macalpine, 2019). These figures highlight the high introduction potential

of willows. Species of the genus *Salix* L. are widespread in the northern and temperate latitudes of the Northern Hemisphere. Willows are absent in the natural flora of Australia, New Zealand, Oceania, Antarctica, eastern Indonesia, and western Africa. The greatest diversity of *Salix* species is found in northern Eurasia, North America, and China (Skvortsov, 1999).

However, under the influence of new habitat conditions, introduced species may undergo certain changes in their nature, their response to factors of the new ecological environment, develop new adaptive mechanisms, and gradually acclimate to different conditions (Bulax & Shumyk, 2013; Yatsyk et al., 2017).To predict the adaptability of an introduced plant to new conditions, researchers compare the climatic and agroclimatic factors of its natural growth regions with those of potential cultivation areas. They also study the species' paleo-range and current distribution, conduct florogenetic analysis, consider past introduction experiences, and examine the plant's response to the most significant ecological factors of the region (Yatsyk et al., 2017).

Since most willows originate from northern and temperate latitudes, the primary limiting factor in expanding their cultivated range southward is drought tolerance. The issue of willow drought resistance remains a focus of many researchers and is widely discussed in the literature. A plant's ability to withstand drought is a combination of its capacity to avoid drought and endure dehydration. In an experimental study on dehydration resistance, Tipton J.L. (2024) examined desert willow, yellow bells, and mulberry, finding that desert willow and yellow bells exhibited higher relative resistance than mulberry in both humid and arid conditions. This suggests that willow leaves experiencing drought stress have adapted to minimize water loss.

In their study on the diversity of willows in Minnesota, J.A. Savage and J.M. Cavender-Bares (2011) compared the survival strategies of willow species found in wet and seasonally dry ecotopes. They determined that species occurring in dry ecotopes exhibit greater physiological divergence.

Chinese researchers investigated the sexual responses to drought and the external application of acetic acid in *S. rehderiana*, *S. babylonica*, and *S. matsudana*, finding that female specimens of these willow species demonstrated greater drought tolerance than male specimens (Xia et al., 2023). Specifically, female willows treated with acetic acid exhibited a more extensive and active root system, higher osmotic and antioxidant capacity, and increased photosynthetic rate. In contrast, male specimens showed a higher presence of reactive oxygen species and greater stomatal closure, indirectly induced by abscisic acid.

The drought tolerance of willows, along with their ability to withstand pollarding and topping, allows for the establishment of forage plantations near farms in New Zealand for use during dry periods (Jones & McIvor, 2013). Drought resistance is also a key criterion for selecting *Salix* species for breeding trials (Macalpine, 2019).

At the same time, the limiting factors restricting the cultivated range of North American, Euro-Siberian, and Far Eastern *Salix* species in Ukraine are high temperatures and low moisture levels – both in the soil and air - during the summer months, which reduce the drought tolerance of these species (Ishchuk, 2016; 2017c). As a result, introduced *Salix* species and their cultivars in Ukraine are primarily found in botanical garden collections, arboretums, and other scientific institutions. However, their presence in urban landscaping is significantly more limited. In the latest edition of the reference book *"Assortment of Trees, Shrubs, and Lianas for Landscaping in Ukraine"* (Kuzneczov et al., 2013), only 11 species and three cultivars are recommended for landscaping in Ukraine: *S. alba* L., *S. alba* 'Vitellina pendula', *S. babylonica* L., *S. acutifolia* Willd., *S. caprea* L., *S. matsudana* Koidz., *S. matsudana* 'Tortuosa', *S. capusii* Franch., *S. caspica* Pall., *S. myrsinifolia* Salisb., *S. cinerea* L., *S. purpurea* L., *S. purpurea* 'Gracilis', and *S. rosmarinifolia* L.

The aim of our research is to identify promising *Salix* species for implementation in landscaping, phytoremediation, and plantation forestry based on an analysis of their natural and cultivated range in Ukraine, as well as their

growth, development, and ecological resilience to environmental conditions.

MATERIALS AND METHODS

The study of the cultivated range of *Salix* L. species was conducted from 2012 to 2022 in the forest, forest-steppe, and steppe natural-climatic zones of Ukraine. The analysis included *Salix* L. species in the collections of botanical gardens and arboretums in Kyiv, Bila Tserkva, Uman, Chernivtsi, Uzhhorod, Lviv, Lutsk, Vinnytsia, Ternopil, Kharkiv, Sumy, Poltava, Dnipro, Kryvyi Rih, Odesa, Kherson, and Zaporizhzhia, as well as in the landscaping of public spaces in these cities (Fig. 1). The general climatic characteristics of Ukraine's natural-climatic zones are presented in Table 1 (Climate…, 2022; Didukh, 2023).

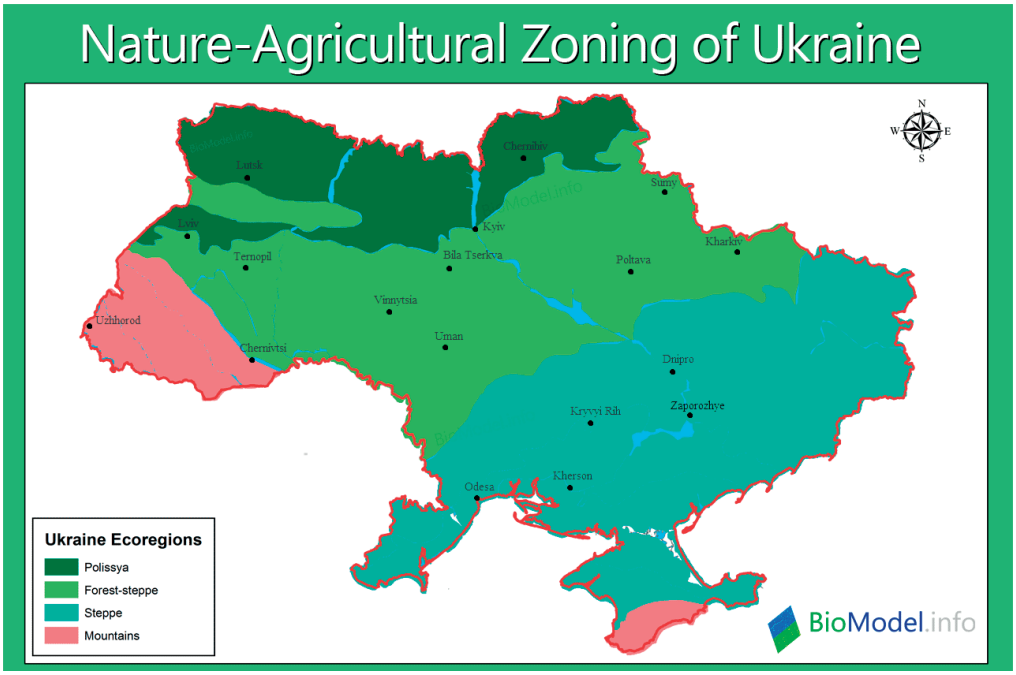


Figure 1. Distribution of introduced *Salix* L. species in Ukraine

Table 1. General Climate Indicators in the Natural-Climatic Zones of Ukraine

Indicator name	Polissya	Forest-steppe	Steppe
Average annual air temperature, ºC	+6.2…+6.6	+8.0…+8.8	+9.0…+10.9
Average January temperature, ºC	-3.0…-6.5	-4.0…-8.0	+1.5…-8.0
Average July temperature, ºC	+17.0…+19.5	+18.0…+20.0	+21.0…23.0
Absolute minimum temperature, ºC	-39.0	-36.0	-40.0
Absolute maximum temperature, ºC	+39.0	+40.0	+41.0
Frost-free period duration, days	160…210	150…190	200…230
Sum of active temperatures above +10 ºC	1940…2580	2500…3400	3450…3550
Soil freezing depth, cm	80…150	90…130	60…160
Vegetation period duration, days	160…180	180…200	200…210
Annual precipitation, mm/year	550…700	450…550	300…450

The subjects of our research were introduced species of the genus *Salix* L., including *S. adenophylla* Hook., *S. alatavica* Kar. ex Stschegl., *S. argyracea* E.L. Wolf, *S. babylonica*, *S. capusii*, *S. caspica*, *S. dasyclados* Wimm., *S. elaeagnos* Scop., *S. integra* Thunb., *S. kangensis* Nakai, *S. matsudana*, *S. miyabeana* Seemen, *S. ledebouriana* Trautv., *S. lucida* Muhl., *S. udensis* Trautv. & C.A. Mey. and *S. schwerinii* A.K. Skvortsov, which are represented in the collections of botanical gardens and

dendrological parks of Ukraine. Additionally, our research included native species such as *S. fragilis* L., *S. myrtilloides* L., *S. lapponum* L., *S. caprea*, *S. aurita* L., *S. starkeana* Willd., *S. purpurea*, *S. vinogradovii* A. Skvorts., *S. viminalis* L., and *S. myrsinifolia* Salisb., which in Ukraine are located at the southern or southeastern limit of their natural range.

The introduction process and all its key stages can generally be divided into three blocks: scientific forecasting or diagnostics, modeling or experimentation, and evaluation of results or the success of species introduction (Bulax & Shumyk, 2013). To implement the first block of the introduction process – scientific forecasting or modeling – we analyzed the geographical origin and soil-climatic conditions of Ukraine and the regions where *Salix* species were introduced. During the second stage, which involves experimental research, we assessed the introduced *Salix* species based on key life activity indicators, including growth, generative development, winter hardiness, and drought resistance. At the third stage of the introduction process, we analyzed the experimental results.

To assess the success of the introduction, we used seven key indicators: the degree of annual shoot maturation, winter hardiness, preservation of plant habitus, ability to form shoots, regularity of shoot growth, capacity for generative development, and methods of reproduction in the introduction region (Kokhno, 1983). Winter hardiness was evaluated using a 7-point scale: I – no damage (25 points); II – frost damage affects less than 50% of the length of annual shoots (20 points); III – frost damage affects 50-100% of the length of annual shoots (15 points); IV – frost damage extends to biennial and older parts of the plant (10 points); V – the crown freezes down to the snow cover level (5 points); VI – the entire above-ground part freezes (3 points); VII – the plant completely perishes (1 point).

The plant habitus was analyzed using a 3-point scale: I– plants retain their natural life form in cultivation, as observed in the wild (10 points); II – plants experience some frost damage, do not restore their above-ground parts to the previous height and volume (5 points); III – plants do not retain their characteristic growth form from nature, as they freeze annually at an early age or exhibit low growth energy (1 point).

The shoot-forming ability was assessed using a 3-point scale: I – high ability (6 or more shoots on a single two-year-old shoot) (5 points); II – moderate ability (3-5 shoots on a single two-year-old shoot) (3 points); III – low ability (2 shoots on a single two-year-old shoot) (1 point). The regularity of shoot growth was evaluated based on the presence or absence of annual increments in the main shoots or branches, considering the plant's age: Annual increment – 5 points; Non-annual increment – 2 points.

The ability of plants for generative development was analyzed using a 4-point scale: I – seeds mature (25 points); II – plants flower, but fruits do not ripen (20 points); III – plants flower, but fruits do not set (15 points); IV – do not flower (1 point). The possible modes of reproduction in the cultivated range were assessed using a 5-point scale: I – self-seeding (10 points); II – artificial sowing (7 points); III – natural vegetative propagation (5 points); IV – artificial vegetative propagation (3 points); V – plants are imported from the natural range (1 point).

In introduction practice and reference literature, the term acclimatization is widely used and interpreted as the adaptation of plants to climatic conditions that differ from those of their natural range. In reality, plants adapt not only to climate but also to soil, hydrological, and other environmental conditions (Kokhno, 1983). To ensure an objective assessment of the success of introduction and the degree of acclimatization of *Salix* species, evaluation criteria were combined and assigned numerical values in the form of a total score – the acclimatization index (Kokhno, 1983). The acclimatization index (A) is the sum of the scores for growth, generative development, winter hardiness, and drought resistance. The highest possible acclimatization index, 100, corresponds to the highest level of introduction success. The formula used to determine the acclimatization index is:

$$A = P \times b_4 + G_3 \times b_2 + W \times b_1 + D \times b_3, \quad (1.1)$$

where (P) is the growth indicator, (G_3) is the generative development indicator, (W) is the winter hardiness indicator, (D) is the drought

resistance indicator, and (b_1-b_4) are the weighting coefficients for each characteristic.

The indicators for growth, generative development, winter hardiness, and drought resistance were visually assessed using five-point scales. The data obtained from visual observations were compared with the weighting coefficient (b), which reflects the significance of each characteristic in the introduction process. The weighting values were determined based on their importance for successful introduction: Winter hardiness – 10, Generative development – 5, Drought resistance – 3, Growth – 2.

The degree of drought resistance in plants was determined using the following scale: good drought resistance under all conditions – 5 points; relative drought resistance (the plant partially sheds leaves during drought) – 4 points; the plant sheds all leaves during drought – 3 points; leaves lose turgor during drought but later recover – 2 points; no drought resistance (the plant dies from drought) – 1 point (Mezhens'kyj, 2007).

Based on the analysis of the viability indicators of willows, the degrees of their acclimatization were determined according to the acclimatization index value: full acclimatization (A = 100), good acclimatization (A = 80), satisfactory acclimatization (A = 60), weak acclimatization (A = 40), no acclimatization (A = 20).

RESULTS AND DISCUSSIONS

In total, the willow flora of Ukraine is represented by 43 species and 11 hybrids of the genus *Salix*, belonging to 20 sections, including 20 introduced species from Siberia, the Far East, Mongolia, China, Japan, and North America (Gorelov, 2002) (Table 2).

Table 2 Natural and Cultivated Range of *Salix* Species Introduced to Ukraine

Species Name	Natural Range	Cultivated Range in Ukraine
S. adenophylla	North America – from Labrador to Wisconsin, south to Pennsylvania and Illinois.	Kyiv, Bila Tserkva, Uman
S. alatavica	Southwestern Altai, Western Mongolia, Sayan Mountains, Tien Shan.	Kyiv, Bila Tserkva, Uman
S. argyracea	Southeastern regions of Kazakhstan, Kyrgyzstan, Xinjiang Uygur Autonomous Region of China.	Kyiv, Bila Tserkva, Uman
S. babylonica	China.	Kyiv, Bila Tserkva, Uman, Chernivtsi, Lviv, Vinnytsia, Ternopil, Odesa, Kharkiv, Poltava
S. capusii	Central Asia.	Kyiv, Bila Tserkva, Uman
S. caspica	Volga region, Ural, Western Siberia, Central Asia, Mongolia, Iran.	Kyiv, Bila Tserkva, Uman
S. dasyclados	Eastern Europe, western, central, and southern regions of Siberia, Far East, Mongolia, and Northern China.	Kyiv, Bila Tserkva, Uman
S. elaeagnos	Western, Southern, and Central Europe. In Ukraine, only in the highlands of the Carpathians.	Kyiv, Bila Tserkva, Uman
S. integra	Japan.	Kyiv, Lutsk, Poltava, Vinnytsia, Bila Tserkva, Uman
S. kangensis	Far East and Southeastern China.	Kyiv, Bila Tserkva, Uman
S. ledebouriana	Siberia, Kazakhstan, Mongolia.	Kyiv, Bila Tserkva, Uman
S. lucida	North America – from Newfoundland west to eastern Saskatchewan, and south to Maryland and South Dakota.	Kharkiv, Bila Tserkva
S. matsudana	China, Korea.	Kyiv, Lviv, Uzhhorod, Chernivtsi, Vinnytsia, Ternopil, Odesa, Uman, Bila Tserkva, Kharkiv, Poltava, Sumy, Dnipro
S. miyabeana	Eastern and Southern Siberia, Far East, Mongolia, and Northeastern China.	Kyiv, Bila Tserkva, Uman, Chernihiv region
S. schwerinii	Southern regions of Eastern Siberia, Far East, southern Kamchatka and Sakhalin, Mongolia and Northeastern China.	Vinnytsia, Kryvyi Rih, Uman, Bila Tserkva
S. udensis	Eastern Siberia, Far East, Southeastern China, Northern Mongolia, Korea and Japan.	Kyiv, Bila Tserkva, Uman

At the same time, it should be noted that these species are unevenly distributed across Ukraine. Ten species (*S. fragilis, S. myrtilloides, S. lapponum, S. caprea, S. aurita, S. starkeana, S. purpurea, S. vinogradovii, S. viminalis, S. myrsinifolia*) are located at the southern or southeastern limit of their range in Ukraine, which is explained by the climatic transition from the northwest to the southeast of the country (Ishchuk, 2014; 2015b; 2017c) (Figure 2).



Figure 2. The southern distribution limit of autochthonous species of the genus *Salix* L. in Ukraine: 1 - *S. lapponum*; 2 - *S. myrsinifolia*; 3 - *S. myrtilloides*; 4 - *S. aurita*; 5 - *S. rosmarinifolia*; 6 - *S. purpurea*; 7 - *S. starkeana*; 8 - *S. viminalis*; 9 - *S. caprea*; 10 - *S. fragilis*

However, isolated occurrences of these species are also found south of their continuous range. In particular, *S. starkeana* has been recorded in Poltava Oblast, 100 km south of its primary range boundary (Ishchuk & Smolyar, 2017), while *S. purpurea* is distributed in the steppe foothills of Crimea (Ishchuk & Ishchuk, 2020). Seven species (*S. herbacea, S. retusa, S. alpina, S. silesiaca, S. rhaetica, S. daphnoides,* and *S. elaeagnos*) have an insular distribution limited to the Ukrainian Carpathians and require conservation efforts (Red Book of Ukraine. Plant World, 2009; Fuchylo & Sbytna, 2009; Ishchuk, 2014; 2017a). According to V.V. Krichfalushiy (1982), *S. alpina* Scop., *S. hastata* L., *S. herbacea* L., *S. kitaibeliana* Willd., *S. lapponum* L., *S. phylicifolia* L. and *S. retusa* L. are rare arcto-alpine and alpine species – relicts of the glacial period – found exclusively in the highlands of the Carpathians. Table 3 presents the geographic distribution of *Salix* species found in Ukraine.

Table 3. Geographical groups of *Salix L*. ranges characteristic of Ukraine

Geographic Groups of Salix Ranges	Distribution in Ukraine
I. Multizonal Species (ranges covering most of the Eurasian continent):	*S. caprea, S. pentandra, S. alba, S. triandra, S. starkeana, S. cinerea*
II. European Species (ranges within Europe)	*S. fragilis, S. aurita, S. viminalis, S. purpurea, S. daphnoides, S. elaeagnos*
III. Eastern European Species (ranges within Eastern Europe)	*S. acutifolia, S. silesiaca*
VI. Eurosiberian Species (ranges covering Northern and Central Europe and Siberia)	*S. rhaetica, S. lapponum, S. myrtilloides, S. myrsinifolia, S. rosmarinifolia, S. vinogradovii*
V. Arctic-Alpine Species (ranges covering Arctic and alpine tundra)	*S. herbacea, S. retusa, S. alpina.*

In Ukraine, the cultivated range includes *S. adenophylla, S. alatavica, S. argyracea, S. babylonica, S. caspica, S. capusii, S. dasyclados, S. integra, S. kangensis, S. ledebouriana, S. lucida, S. matsudana, S. miyabeana, S. udensis* and *S. schwerinii*.

However, with the exception of the garden forms *S. integra* 'Hakuro-Nishiki', *S. integra* 'Pendula' and *S. matsudana* 'Tortuosa', most species are represented only by individual specimens in botanical garden and arboretum collections and are nearly absent from urban landscaping. The largest and oldest willow collection in Ukraine is housed at the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine in Kyiv, comprising 45 species, hybrids, and forms (Kruglyak et al., 2009). Most willow collections are concentrated in the forest-steppe zone of central Ukraine, including the Botanical Garden of Bila Tserkva National Agrarian University, the State Dendrological Park "Alexandria" of the National Academy of Sciences of Ukraine (Bila Tserkva), the Sofiyivka National Dendrological Park of the National Academy of Sciences of Ukraine (Uman), the Kryvyi Rih Botanical Garden (Kryvyi Rih), the Berezne State Dendrological Park in Rivne region and the "Ivushka" Arboretum in Poltava region (Ishchuk, 2019).Typically, in botanical gardens, *Salix* collections are planted on gentle slopes as a single generic complex, creating uniform edaphic and microclimatic conditions within the plot. This approach allows for the study of all introduced species under relatively similar environmental conditions (Kruglyak et al., 2009; Ishchuk, 2013a; 2018).

The introduction trials in the Forest-Steppe zone of Ukraine also included *S. aurita, S. elaeagnos, S. lapponum, S. myrsinifolia,* and *S. myrtilloides*, whose natural range is limited to the northern and western parts of Ukraine, primarily within the Polissya zone (Ishchuk, 2015a; 2017b).

To assess the success of introductions in the cultivated range, evaluations were conducted on the willow species' life form, annual shoot growth, and degree of lignification by the end of the growing season, as well as their winter hardiness, drought resistance, and methods of propagation in cultivation.

When transferred to the cultivated range, *S. argyracea, S. schwerinii, S. udensis* and *S. kangensis* exhibited a change in life form from "tree" to "shrub." Meanwhile, *S. capusii* and *S. caspica*, which naturally grow as small trees or shrubs in their native habitat, formed only the "shrub" life form in Ukraine (Table 4). The remaining introduced species did not undergo changes in life form.

Table 4. Results of the evaluation of the introduction success of *Salix* species using an integrated scale in Ukraine

Species	Life form:		Age of Plants (Years)	Winter Hardiness Score	Viability Indicators (Points):							Overall Assessment:	
	in the wild	in cultivation			Shoot Lignification	Winter Hardiness	Shape Retention	Shoot Formation	Height Growth	Possible Methods of Preservation in Cultivation	Possible Methods of Propagation in Cultivation	Total Viability Indicators Score	Prospect Group
S. aurita	shrub	shrub	1–10	1	20	20	10	3	5	25	3	87	I
S. adenophylla	shrub	shrub	1–10	1	20	20	10	3	5	25	3	87	I
S. alatavica	shrub	shrub	10	1	20	25	10	3	5	25	3	97	I
S. argyracea	tree	shrub	1–10	1	20	25	10	3	5	25	3	89	I
S. babylonica	tree	tree	1-60	2	15	20	10	3	5	25	3	80	I
S. capusii	small tree-shrub	shrub	1–10	1	20	25	10	3	5	25	3	89	I
S. caspica	small tree-shrub	shrub	1–10	1	20	25	10	3	5	25	3	89	I
S. dasyclados	shrub	shrub	1–10	1	20	25	10	3	5	25	3	91	I
S. elaeagnos	tree	tree	1–10	2	15	20	10	3	5	25	3	83	I
S. integra	tree	tree	10	2	15	20	10	3	5	20	3	78	I
S. kangensis	tree	shrub	10	1	20	25	10	3	5	20	3	87	I
S. lapponum	shrub	shrub	1–10	1	20	20	10	3	5	25	3	87	I

S. matsudana	tree	tree	1-10	2	15	20	10	3	5	20	3	75	I
S. miyabeana	shrub	shrub	1-20	2	15	20	5	3	5	25	3	78	II
S. myrsinifolia	shrub	shrub	1–10	1	20	20	10	3	5	25	3	87	I
S. myrtilloides	shrub	shrub	1–10	1	20	20	10	3	5	25	3	87	I
S. ledebouriana	shrub	shrub	1–10	1	20	25	10	3	5	25	3	91	I
S. lucida	tree	tree	1–8	1	20	20	10	3	5	25	3	87	I
S. schwerinii	tree	shrub	1–10	1	20	25	8	3	5	25	3	90	I
S. udensis	tree	shrub	10	1	15	15	8	3	5	25	3	75	II

The frost resistance score was evaluated based on the degree of lignification of the shoots. Incompletely lignified shoots by the end of the growing season were recorded in *S. integra*, *S. elaeagnos*, *S. babylonica*, *S. matsudana* and *S. miyabeana*, which indicates a lower frost resistance of these species. In their natural habitat, they have a longer growing period (Ishchuk, 2018). The low degree of frost resistance of *S. matsudana* in the conditions of Western Ukraine was also confirmed by the researchers of the Botanical Garden of the National Forestry University of Ukraine in Lviv (Melnyk et al., 2010).

The process of shoot formation and growth in all the studied species within their natural and cultivated ranges remained unchanged. All the studied *Salix* species in the cultivated range in Ukraine propagate through lignified cuttings. At the same time, while studying the energy properties of willows, scientists established that the annual growth of willows and the number of shoots per shrub, including *S. dasyclados*, depend on planting density and the amount of nitrogen fertilizer applied in the plantation (Sinchenko et al., 2022). Our studies of *S. dasyclados*, *S. caspica*, *S. capusii*, *S. adenophylla*, *S. argyracea*, *S. ledebouriana*, *S. schwerinii* and *S. udensis*, which are also promising species for energy plantations, fully confirm these findings.

Based on the sum of viability indicators, all the studied species in the cultivated range in Ukraine, with the exception of *S. miyabeana* and *S. udensis*, belonged to the highest first group of introduction prospects.

When determining the acclimatization level for each introduced willow species in Ukraine, growth, generative development, frost resistance, and drought tolerance indicators were analyzed. By analyzing the average annual growth, we recorded a wide range of values, from 15 cm (*S. alatavica*, *S. lapponum*) to 103 cm (*S. dasyclados*) (Figure 3). This can be explained by the fact that the studied willow species in their natural habitats also exhibit different growth energies, especially in small shrub forms with a compact crown structure, such as *S. lapponum*, *S. miyabeana*, *S. myrsinifolia* and *S. myrtilloides*. However, the annual growth range for most species fluctuates between 50 and 100 cm, ensuring the preservation of a similar plant habitus in both natural and cultivated ranges.

The expansion of the cultivated range of *Salix* species in Ukraine, particularly under global climate change, is most influenced by the drought tolerance of willows. This trait is a limiting factor for increasing the diversity of willows in the southern and eastern regions of Ukraine (Gorelov, 2016; Ishchuk, 2016; 2017c). However, most researchers agree that *Salix* species have a certain potential for drought tolerance. Under artificial drought stress conditions, *S. myrsinifolia* seedlings were more susceptible to drought stress than hybrids of *Salix myrsinites L.* × *Salix myrsinifolia* (Turtola et al., 2006). According to Polish researchers, *S. viminalis* is characterized by high drought tolerance (Drzewiecka, 2023), and in Ukraine, it also has insular habitats outside its continuous range in the Odesa region. At the same time, in studies by Chinese scientists, *S. babylonica* was found to be less drought-tolerant than the desert willow *Salix gordejevii* (Yang et al., 2004).

The drought tolerance of willows is also significantly influenced by the origin of the species. According to our observations in the cultivated range, the most drought-tolerant species are those of southern origin from the Volga region, southern Siberia, Central Asia, Iran, Mongolia, and China, including *S. capusii*, *S. caspica*, *S. babylonica*, *S. miyabeana*, *S. ledebouriana* and *S. schwerinii*,

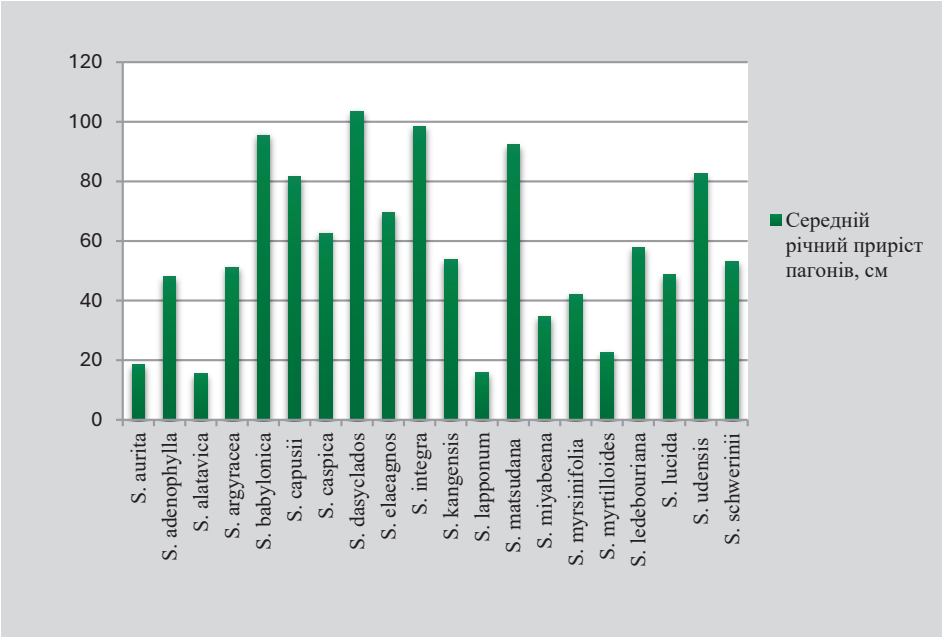


Figure 3. Average annual shoot growth of *Salix* species introduced to Ukraine

as confirmed by studies conducted in Kyiv by O. Gorelov (2016). At the same time, despite its origin from Korea and China, *S. matsudana* was found to be less drought-tolerant in our studies. Our findings on the drought tolerance of *S. matsudana* align with experimental research conducted in Canada (Doffo et al., 2016).

Salix species are dioecious plants with unisexual flowers arranged in catkins. They bloom depending on the species – before leaf bud break, simultaneously with it, or after the leaves have fully unfolded. In the cultivated range in Ukraine, only three willow species (*S. argyracea*, *S. miyabeana*, *S. udensis*) bloomed but did not produce fruits or seeds (Table 5). The other species regularly bloomed in Ukraine, and we recorded the first flowering of trees and shrubs propagated vegetatively at the age of 4-6 years.

Table 5. Degree of acclimatization of introduced *Salix* species in Ukraine

Species	Indicators				Acclimatization Index	Degree of Acclimatization
	growth	generative development	frost resistance	drought resistance		
S. aurita						
S. adenophylla	10	25	50	10	95	full
S. alatavica	10	25	50	10	95	full
S. argyracea	10	15	50	10	85	good
S. babylonica	10	25	40	15	90	full
S. capusii	10	25	50	15	100	full
S. caspica	10	25	50	15	100	full
S. dasyclados	10	25	50	10	95	full
S. elaeagnos	10	25	40	10	85	good
S. integra	10	25	40	10	85	good
S. kangensis	10	25	50	10	95	full
S. lapponum	10	25	50	10	85	good
S. ledebouriana	10	25	50	15	100	full
S. lucida	10	25	50	10	95	full

S. matsudana	10	25	30	10	75	good
S. myrsinifolia	10	25	50	10	75	good
S. myrtilloides	10	15	50	10	85	good
S. miyabeana	10	15	40	15	80	good
S. schwerinii	10	20	50	15	95	full
S. udensis	10	15	50	10	85	good

According to the acclimatization index, which includes the sum of integral indicators for growth, generative development, frost resistance, and drought tolerance, nine species (*S. argyracea*, *S. elaeagnos*, *S. integra*, *S. matsudana*, *S. myrsinifolia*, *S. myrtilloides*, *S. miyabeana*, *S. lapponum*, *S. udensis*) have demonstrated good acclimatization in the forest-steppe zone of Ukraine, while the remaining species have fully acclimatized to the new soil and climate conditions. A decade of experience with the cultivation of introduced willows in Ukraine has shown that species from the eastern and northern regions of North America, Siberia, Mongolia, the Far East, Japan, and northern China are most effectively optimized for introduction (Ishchuk, 2013b; 2015a).

CONCLUSIONS

As long-term experience with *Salix* cultivation in Ukraine shows, introduced species of the *Salix* genus differ in their geographic origin and exhibit varying degrees of introduction success. Ukraine cultivates twenty introduced willow species, with the greatest diversity represented in scientific collections in cities of the forest-steppe zone – Kyiv, Bila Tserkva and Uman. Most of these species have fully acclimatized to the new conditions, except for *S. argyracea*, *S. elaeagnos*, *S. integra*, *S. lapponum*, *S. matsudana*, *S. myrtilloides*, *S. miyabeana* and *S. udensis*, which are characterized by a good degree of acclimatization.

For southern-origin species such as *S. integra*, *S. elaeagnos*, *S. babylonica*, *S. matsudana* and *S. miyabeana*, delayed shoot maturation is characteristic, which leads to reduced frost resistance. However, these species exhibit a high ability to form shoots, which aids in the rapid restoration of their habitus. Six species – *S. argyracea*, *S. capusii*, *S. caspica*, *S. kangensis*, *S. schwerinii* and *S. udensis* – under cultivated conditions change their life form from “tree” or “small tree” to “shrub”.

The limiting factors that restrict the cultivated range of Euro-Siberian and Far Eastern *Salix* species in Ukraine are high air temperatures and low soil moisture in summer. These conditions lead to growth suppression, reduced leaf blade size, temporary loss of leaf turgor, and overall decreased ornamental value.

Promising regions for expanding the biodiversity of willows in Ukraine are areas with a temperate climate, such as North America, as well as Siberia, the Far East, Mongolia, Japan, and northern China.

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RESEARCH ON THE USE OF MOLECULAR MARKERS TO IDENTIFY THE *NPTII* GENE INVOLVED IN GENETIC MUTATION PROCESSES

Ioana KLEE, Lenuţa CHIRA, Daniel NAGY, Constantin PĂUN, Ligia ION

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: ionnagyligia@yahoo.fr

Abstract

Because, for medicinal and aromatic plants there is the ease of being genetically modified both naturally through mutations and through external intervention. In this context, the effectiveness of the present protocol to produce good quality DNA suitable for the detection of genetically modified crops was evaluated. Genomic DNA isolated from different parts of coriander, fennel and sage plants through was done. For this, through the PCR technique, three molecular markers were used to amplify the neomycin phosphotransferase gene (nptII), which is used as a marker gene in mutation identification processes. The existence of nptII (target of 173 bp) was investigated in the plant material studied in the 3 plant species (coriander, fennel and sage), vegetative parts (leaves) but also the seeds considered in this work. Successful PCR amplification of the nptII gene with a randomly amplified polymorphic DNA primer and complete digestion of the isolated DNA with the restriction enzyme HindIII validated the quality of the isolated DNA.

Key words: *Salvia officinalis, genomic DNA, mutations, Coriandrum sativum, Foeniculum vulgare, genes.*

INTRODUCTION

The efficient selection of plants modified by natural mutations as well as transgenic plants is essential in plant biotechnology and plant exploitation. For this reason, the development of efficient, specific marker systems plays a crucial role in identifying genes involved in these complex processes. The evaluation of an improved spectinomycin resistance construct to provide strong selection in transformation processes has been highlighted (Eustice et al., 1983; Jefferson et al., 1987). The new construct was compared, in several plant species, with the standard selectable spectinomycin marker gene currently used for the transformation of soybean and other medicinal, aromatic and crop plants and with the neomycin phosphotransferase (*nptII*) gene that confers resistance to kanamycin. La *Arabidopsis thaliana*, potato and citrus, the transformation efficiency provided by the improved spectinomycin marker was approximately equal to that of kanamycin and considerably better than that observed for the standard spectinomycin marker. (Miki et al. 2004, Dekeyser et al.1989). For soybeans, the improved spectinomycin resistance marker performed as well as the standard spectinomycin gene. These results demonstrate that the improved spectinomycin construct provides strong resistance comparable to kanamycin in several species, offering an alternative tool for the selection of transgenic and mutated plants (Nitovska et al., 2015; Guerche et al., 1987). Antibiotic and herbicide resistance genes are the most effective and widely used selectable markers (Miki et al., 2004). The *hygromycin phosphotransferase-II* (*hptII*) gene is the most used antibiotic selectable marker in genetic transformation and mutation detection in various plants. However, the antibiotic hygromycin that selects transgenic or mutated plants expressing the *hptII gene* is expensive in the setting of developing countries. The other gene most widely used for selection of transgenic and mutated plants is *neomycin phosphotransferase*-II (*npt II*) (Martinell et al., 2013).

The *neomycin phosphotransferase-II* gene has been used as a selection marker in many commercialized transgenic plants as well as those that have undergone natural or chemical mutations and may face fewer regulatory hurdles for future commercialization of transgenic crops. *Neomycin phosphotransferase-II* has been approved by the U.S. Food and Drug Administration (FDA) as a food additive for tomatoes, cotton, and canola

(U.S. Food and Drug Administration, FDA in 1994). The *nptII* gene encodes the enzyme aminoglycoside 3′-phosphotransferase and is inactivated by phosphorylation by several aminoglycoside antibiotics such as neomycin, kanamycin, paromomycin, ribostamycin, butyrosin and geneticin (G418). However, native rice callus has been observed to exhibit varying degrees of natural innate resistance to kanamycin (Caplan et al., 1992). Selection on G418 is more effective than kanamycin for transgenic or mutated plants expressing the *nptII* gene, probably because G418 is more toxic than kanamycin to wheat, rice and soybean cells (Twyman et al., 2022). Chan et al., 1992, used G418 for Agrobacterium-mediated transformation of various plants, however, following selection on 40 mg l^{-1} of G418, a total of only four transgenic plants were produced.

Also, two aminoglycosides, G418 and paromomycin, were used for sweet potatoes to develop a reliable selection system for both mutated and transgenic plants (*Ipomoea batatas* (L.) Lam.) (Jones, 1980; Shin YM et al., 2007). For soybean plants, genetic transformation is limited by the lack of multiple efficient, specific marker systems. The most commonly used soybean transformation relies heavily on selection with hygromycin phosphotransferase II (*hptII*). Transgenic events were detected using *nptII* with enhanced G418 selection without generating contamination. Six independent, fertile transgenic plants were identified using *nptII* and G418, a frequency similar to that obtained with hygromycin selection. Soybean embryogenic cultures co-transformed with *hptII* and *nptII markers* demonstrated resistance to both hygromycin B and G418, while plant regeneration and fertility were not adversely affected. *nptII* has been useful as a second selectable marker for multiple genetic transformations in basic and applied soybean research. (Li R. et al., 2011). Also, for medicinal and aromatic plants, there is the easiness of being genetically modified both naturally through mutations and through external intervention. (Wang et al., 2011). The applications of PCR technology are very diverse, starting from those aimed at identifying, characterizing or controlled modification of genes using as a template either directly purified DNA molecules from various sources, or cDNA molecules derived from reverse transcription of the mRNA of interest. The applications have also developed in practice, especially in the horticultural field, for diagnosing harmful diseases by detecting the pathogens that cause them. (Ion et al., 2019; Church et al., 2012; Dilhari et al., 2017; Don et al., 1991; Dellaporta et al., 1983).

MATERIALS AND METHODS

The plant material consisted of plant and seed samples from the species common sage (*Salvia officinalis* L.), coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare),* originating from the Buzău Research Station, and the fennel samples from the Bucharest Botanical Garden.

Molecular research began with the classic genomic DNA isolation protocol following the CTAB method of Doyle and Doyle (1987) for the species, common sage (*Salvia officinalis* L.), coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare).*

DNA concentration was determined spectrophotometrically at an absorbance of 260 nm (A260) using NanoDrop1000 (Thermo Scientific). The purity of DNA from protein and polysaccharide contamination (Reinoso et al., 2016) was assessed by reading the absorbance ratio at A260/A280 and A260/A230, respectively. The quality of the DNA extract was also assessed by electrophoretic separation for all DNA samples from all 3 species studied and also on particular parts of the plants evaluated, respectively the aerial part and seeds. For this, a 2% agarose gel was prepared, stained with ethidium bromide (1 µg/ml). Standard protocol isolation, genomic DNA according to the CTAB method of Doyle and Doyle (1987) involves the following work steps: grinding in liquid nitrogen approximately 0.5 g of young leaves/sample until a fine powder was obtained. The powder thus obtained was transferred to Eppendorf tubes, with 700 µl of extraction buffer (maintained at 6°C); after which the tubes were inverted several times. The samples were then placed on a water bath at 65°C for 25 minutes, inverting the tubes to ensure a constant temperature. After the water bath (Bain Marie), the tubes were allowed to cool to room temperature. Then 700 µl of chloroform: isoamyl alcohol (24:1) was added,

homogenization was performed by consecutively inverting the tubes 20-25 times, until a homogeneous emulsion was obtained. Centrifugation was done at 12,000 rpm for 15 minutes. The aqueous phase (above) was transferred to another sterile tube. If the aqueous phase is cloudy, it is recommended to repeat the chloroform: isoamyl alcohol extraction (steps 4-6). 0.5 volumes of NaCl were added to the solution recovered in the previous step and the contents of the tubes were homogenized. 2 volumes of 95% ethyl alcohol kept in the freezer at -20°C were added and the tubes were placed in the refrigerator at 4-6°C for 15-20 minutes, until the DNA filaments became visible in solution. Centrifugation was performed at 3,000 rpm for 3 minutes, after which the speed was increased to 12,000 rpm for another 5 minutes. The supernatant was carefully removed without detaching the DNA pellet from the walls or bottom of the Eppendorf tube. 700 µl of 76% ethyl alcohol was added and kept at 0-4°C. A new centrifugation at 10,000 rpm for 5 minutes followed, after which the supernatant was carefully removed. The opened tubes were placed in a speed-vac until the alcohol evaporated , approximately 20-30 minutes. The DNA pellet was redissolved in 50 µl of TE buffer solution. The genomic DNA thus extracted from leaf tissue collected from sage, fennel and coriander plants was used by PCR technique to amplify the 575 bp region of *the nptII fragment by using nptII* specific primer sequences:

P52- 5`- CCG GTG TCT G - 3`

P53 - 5`- TGC ATT CCG A - 3`

P54 - 5`- TGC ATT CCG A - 3`

Each PCR reaction was performed in 25 µL (total volume) of reaction mixture, comprising 100 ng of template DNA, 0.2 µL (1 unit) of Taq DNA polymerase (MBI Fermentas, UK), 2.5 µL of 10X PCR buffer (MBI Fermentas, UK), 2.5 µL of 2 mM dNTP mix (MBI Fermentas, UK), 0.5 µL of forward primer and reverse primer (10pM/µL) each, and the volume was completed using molecular grade double-distilled water. The thermal cycling conditions used comprised 3 min at 94°C for initial denaturation, followed by 35 cycles of 94°C for 30 s, 58-62°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min, finally stopping the reaction at 4°C. The PCR protocol was performed using the Mastercycler (EP Gradient Eppendorf). DNA from a transgenic plant was included in the experiments as a control. *The nptII* amplified DNA fragments were separated by electrophoresis on a 1.2% agarose gel, detected by ethidium bromide staining, visualized, and documented on the Molecular Imager Gel Doc XR system (Bio-Rad Laboratories, CA, USA).

RESULTS AND DISCUSSIONS

The DNA yield obtained with the CTAB method of Doyle and Doyle (1987) was 450.2 ng DNA/ µl, of dried leaf tissue and 480.4 ng DNA/ µl for samples obtained from seeds for the Coriander species, and the absorption ratios of 260/280 and 260/230 nm were on average 1.78, 1.45 for plant samples and 1.98 and 1.45 for seed samples, respectively, revealing slight contaminations in the case of coriander. It is observed that the samples obtained from seeds have a higher amount of DNA than those obtained from leaves (Ion et al. 2018). For the *Fennel species,* the amount of DNA was lower, 344.2 ng DNA/ µl, of dry leaf tissue and 485.1 ng DNA/ µl for samples obtained from seeds, and the absorption ratios of 260/280 and 260/230 nm were on average 1.88, 1.85 for plant samples and 1.51 and 1.50 for seed samples, respectively, revealing slight contaminations.

Table 1. Results regarding the quantity of genomic DNA isolated from coriander, sage and fennel species using the standard protocol CTAB method of Doyle and Doyle (1987)

Plant genotypes	Parameters			
	Quantity ng/µl dsDNA	R260/280 (DNA/protein >1.7)	R260 /230 (Leftovers alcohol <1.5)	320 (Polysaccharides -contaminations <0.1)
Coriander-plant	450.2	1, 78	1.45	0.100
Coriander-seed	480.4	1.98	1.45	0.021
Fennel - plant	344.2	1.88	1.51	0.053
Fennel - seed	485.1	1.85	1.50	0.049
Salvia officinalis - plant	389.5	1.92	0.89	0.076
Salvia officinalis-seed	552.2	1.89	1.35	0.013

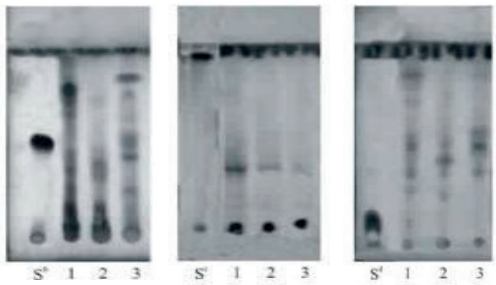
For *the Salvia officinalis species*, the amount of DNA was quite uneven with a large difference between the two types of samples (plant and signs), of 389.5 ng/ µl DNA/ µl, of dry leaf tissue and 552.2 ng DNA/ µl for the samples obtained from the signs and the absorption ratios of 260/280 and 260/230 nm were on average 1.92, 1.89 for plant samples and 0.89 and 1.35 for seed samples, respectively, achieving a difference of 162.7 ng DNA/ µl.

For all three species, it was observed that seeds are the parts of the plants richest in genetic material.

Since medicinal and aromatic plants are prone to genetic modification both naturally through mutations and through external intervention, in this context, the effectiveness of the present protocol to produce good quality DNA suitable for the detection of genetically modified or mutated crops was evaluated. PCR analyses performed on genomic DNA isolated from sage, fennel and coriander plants confirmed the presence of the *nptII* gene in the genome of fennel plants and in the presence of the P53 marker (Figure 2).

Table 2. Results regarding the structure of primers used in DNA amplification

Primer	Primer name	Primer sequence	Molecular weight
P 52	PC 12	5`- CCG GTG TCT G - 3`	3124.1 g/mol
P53	Pf 2.3	5`- TGC ATT CCG A - 3`	2994.2 g/mol
P 54	Pso 3.6	5`- CTG TAC CCC G - 3`	3222.4 g/mol



A - Primer P52 b- Primer P53 c- Primer P54

Figure 1. Migration of DNA fragments in coriander, fennel and sage species for Primers P52, P53, P54



Figure 2. Results of PCR product analysis

The primer sequences were

P52- 5`- CCG GTG TCT G - 3`

P53 - 5`- TGC ATT CCG A - 3`

P54 - 5`- TGC ATT CCG A - 3`

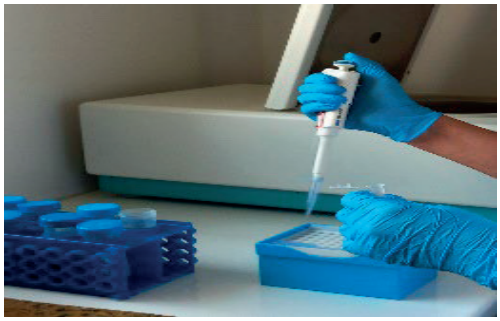


Figure 3. DNA isolation from coriander, fennel and sage

The existence of *nptII* (size 173 bp) was investigated in the plant material taken into study in the 3 plant species (coriander, fennel and sage) vegetative parts (leaves) but also the seeds considered in this work. For this, specific primers were used, following which of them are able to highlight the presence of the gene. Primers P52, P53 and P54 amplified fragments of 575 bp, 478 bp and 175 bp, proving the presence of the *nptII* gene only primer P 53 in the sampled genomes. Taken together, the PCR experiments and the genomic DNA of the presumed plants that underwent mutations confirmed the existence of the gene *nptII* in the genome of the fennel genotypes.



Figure 4 Results regarding the identification of the *nptII gene* in the three species sage, fennel and coriander

The results obtained from electrophoresis demonstrate the presence of the *nptII gene* in the fennel species with a visible band in the agarose gel. The results validate the molecular markers used and the reasoning refers to the source of the plant material procured from the botanical garden and not from a controlled culture.

Intact DNA resulted in bands very close to the edges (Figure 4, upper band), indicating high purity of the extracted DNA, without RNA contamination, especially since the recommended and most accurate way to determine RNA contamination is to run a sample on an agarose gel where another RNA band, if present, will be visible in the gel. Therefore, the higher absorbance ratios A260/A280 in our case can be attributed to slight changes in the pH of the extracted samples (Williams J.G., 1990). A polysaccharide contamination was also assessed (Table 1) by estimating the absorbance ratio A260/A230 as a secondary measure of nucleic acid purity. This ratio is important to assess the level of salt residues in the purified DNA. It is recommended to be greater than 1.5 and preferably close to 1.8. (Tamari et al., 2013; Valentini et al., 1996).

CONCLUSIONS

The effectiveness of the protocol proves its suitability for other applications for food safety assessment by detecting naturally mutated or genetically modified (GM) crops and constitutes a valuable practice in biodiversity conservation. Isolation of quality DNA from sage, fennel and coriander plants is an essential step for satisfactory results in molecular studies, especially those involving plant genetics. For all three species, it has been observed that the seeds are the plant parts richest in genetic material.

The DNA isolation method used in this work has proven to be successful and applicable for DNA extraction with high yield and purity for the 3 different plant species *Salvia officinalis, Foeniculum vulgare and Coriandrum sativum.* The DNA extracted through the standardized protocol gave detectable results and reproducible bands for the detection of the *npt* II gene (173 bp target) for PCR amplification with the ultimate goal of identifying naturally mutated or genetically modified crops. The A260/A280 purity ratio is an important measure for estimating the levels of polyphenol contamination of the extracted DNA. The primer that succeeds in detecting the *nptII gene* is the primer P53- 5`- TGC ATT CCG A - 3`. The target fragments can be amplified by electrophoresis in 1.2% agarose gel. The present work has achieved its goal of developing a protocol for identifying plants that may undergo mutations in technological processes or organisms that have been genetically modified, especially since aromatic and medicinal plants can constitute the raw material for nutritional supplements. Continuing research in this field is important for the development of new applications and the improvement of existing techniques capable of exploring and better understanding the complexity of biological molecules.

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THE EFFECT OF GROWTH STIMULANTS ON THE ROOTING OF CUTTINGS OF PLANTS OF THE GENUS *COTONEASTER* MEDIC. ON DIFFERENT SUBSTRATES

Tetiana KOPYLOVA, Volodymyr HRABOVYI, Olha POROKHNIAVA, Yuriy RUMIANKOV, Alla KONOPELKO, Larisa NOVAK

National Dendrological Park 'Sofiyivka' of the National Academy of Sciences of Ukraine, 12a Kyivska Str., Uman, Ukrain

Corresponding author email: kt.pyracantha@gmail.com

Abstract

The results of studies on the regeneration ability of green and semi-woody cuttings of plants of the genus Cotoneaster Medic. for obtaining high-quality planting material are presented. The effect of plant growth stimulants (Heteroauxin, Epin +, Kornevin, Charkor) on rooting and biometric indicators of cuttings in protected soil conditions in different substrates peat + sand 2:1 (pH 4.8), sand (pH 5.7), peat substrate Mixkar MKS-10-6 (pH 5.2-6) was analyzed. The object of the study was represented by five species of Cotoneaster - Cotoneaster ascendens Flink & B. Hylmö, Cotoneaster hjelmqvistii Flinck & B. Hylmö, Cotoneaster horizontalis Decne., Cotoneaster nan-shan M.Vilm. ex Mottet, Cotoneaster dammeri C.K.Schneid, which are characterized by high decorativeness and are in demand, which requires maximum efficiency of vegetative propagation and cultivation in the conditions of the Right-Bank Forest-Steppe of Ukraine. Therefore, finding new technologies and improving existing ones for vegetative propagation of species of genus Cotoneaster is an extremely urgent task that requires a scientific solution.

Key words*: vegetative propagation, cuttings, biometric indicators, growth regulators, substrate.*

INTRODUCTION

The polymorphic genus *Cotoneaster* (Medic.) Bauhin contains 175 taxa, according to Flinck and Hylmö (1966). In his work "Sinopsis der Gattung Cotoneaster Medicus I", Klotz (1982) included 230 taxonomic units in this genus (Grevtsova, 1999). Recently, Plants of the World Online (POWO) accepted 268 species in the world flora (POWO, 2025).

The range of most species is located in the mountainous regions of Central Asia, Iran, Afghanistan, India, Mongolia, and China. In Western European countries, they have been used in landscaping since 1825 (Dickoré et al., 2010). Three species of *C melanocarpus*, *C. integerrimus* and *C. horizontalis* occur naturally in Ukraine (Grevtsova & Drabinyuk, 2020). *Cotoneaster* cultivars are diverse plants in terms of decorative characteristics, which helps create a desirable composition in gardens, parks, and home areas. They differ in the variety of habit, height, shape, leaf arrangement, abundance of flowering and fruiting, autumn leaf color, and are able to tolerate pruning well (Meng et al., 2021; Fryer & Hylmö , 2009; Grevtsova, 1999). *Cotoneasters* are of particular value as highly decorative plants in the autumn period, when plantings lack bright colors. At this time, their bushes are covered with orange, purple, dark red, black, pear-shaped, rounded fruits, which are placed on the shoot singly, in pairs, or as a raceme. They are used in border plantings, alpine gardens, as ground cover plants, and green fences. The largest collection in Ukraine was created in the O.V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv, which consists of 200 taxa (Grevtsova et al., 2016; Grevtsova et al., 2017). The introduction of plants of the genus *Cotoneaster* in the National Dendrological Park "Sofiyivka" of the NAS of Ukraine began in 1959, and currently the collection is represented by 20 taxa.

Currently, the reproduction, growth, and use of deciduous and evergreen shrubs is extremely relevant and is discussed in the works of scientists in various fields of activity: biologists, dendrologists, ecologists etc.

In Ukraine, studies of the influence of cutting timing and growth stimulants on the rooting of green and semi-woody cuttings of ornamental shrubs using various substrates were conducted by various researchers.

In particular, Grevtsova and Kazanskaya (1997) studied the propagation of green, semi-woody and woody cuttings in cold greenhouses of the most decorative *Cotoneaster* species. Experiments conducted by the authors on the propagation of *C. megalocarpus, C. lucidus, C. przewalskii, C. roseus* by green cuttings on a substrate of sand + peat revealed differences between species and treatments. Maximum yield of rooted cuttings of *C. megalocarpus* was 40%, when using dimethyl sulfoxide (DMSO) 0.001%, same as the control; 100% at *C. lucidus* when using maleic acid hydrazide (HMA) 0.001%, compared to 64% in the control; 84% at *C.przewalskii* when using dimethyl sulfoxide (DMSO) compared to 60% in the control and 84-86% at *C. roseus* when using HMA and DMSO, compared to 38-48% in the control. Semi-woody cuttings were also rooted better when stimulants were used.

Tokman (2020) researched and developed measures and techniques for accelerated propagation of *Ligustrum vulgare* based on stem cuttings in the conditions of the northeastern Forest-Steppe of Ukraine. The optimal period for harvesting cuttings coincides with the dormant period of plants, when the rooting rate was 97%. Optimal for vegetative propagation of *Ligustrum vulgare* are three- and four-node cuttings, which significantly outperform two-node cuttings in terms of growth and development. An effective stimulator of regeneration processes in *Ligustrum vulgare* cuttings was Rhizopon AA powder (the rooting rate was 97%).

Kovalchuk et al. (2021) determined the percentage of engraftment and growth rates of the most common evergreen trees and shrubs in the Volyn region that are propagated by cuttings. Among the studied forms of evergreen plants, the highest percentage of rooting was observed in *Thuja occidentalis* 'Ericoides' (92%), *Thuja occidentalis* 'Aurea' (89%), *Buxus sempervirens* L. (94%) and *Thuja occidentalis* 'Globosa' (83%). The lowest percentage of rooting was observed in *Thuja occidentalis* 'Columna' (65%). Among trees, the greatest growth of the underground part was observed in *Picea pungens* 'Glauca' (26 cm) and among shrubs, in *Juniperus communis* L. (17 cm) and *Juniperus sabina* L. (13 cm).

Kopylova (2021) performed vegetative propagation of plants of the genus *Pyracantha* by rooting green, semi-woody, and woody cuttings, grafting, and also by the method of horizontal burial of shoots in the soil. The best indicators of cuttings' rooting were obtained in fine moisture conditions when planting semi-woody cuttings in a peat substrate KST (2:1:1) and green cuttings using heteroauxin 100 mg/l. Omelyanova and Kotovska (2021) conducted experiments on rooting cuttings of various types of citrus crops on such substrates as peat, sand, coconut substrate, vermiculite, peat with the addition of sand, coconut substrate with the addition of vermiculite. According to the results the highest percentage of survival was observed when growing plants in coconut substrate with the addition of vermiculite (85–98%). Planting material obtained by green cuttings using coconut substrate with the addition of vermiculite or vermiculite alone showed high rooting results.

Mikulich et al. (2023) experimentally investigated the effect of phytohormonal preparations on the rooting of cuttings and the effect of irradiation with red and blue lasers on cuttings of *Buxus sempervirens.* According to the results the positive effect was provided by complex irradiation with red and blue lasers with the longest duration of irradiation. All growth stimulants used during the study had a positive effect on the processes of root formation, but the greatest effect was achieved by "Heteroauxin" - 90%.

Pantsyreva et al. (2024) studied the rooting of green cuttings of the Viburnum 'Velikoplodna' variety. The rooting and subsequent growth and development of rooted three-node cuttings significantly outweigh the similar indicators of two- and one-node cuttings. Three-node cuttings harvested from the apical part showed the best rooting – 53.5%.

Poşta and Bernardis (2018) carried out research on vegetative propagation of *Cotoneaster dammeri* 'Skogholm' This study had as object the simultaneous influence of both nutritive mixture and foliar fertilizers on the growth and development of the seedlings The general conclusion was that vigorous *Cotoneaster dammeri* 'Skogholm' seedlings were obtained by using a nutritive sublayer composed of 30%

manure; 20% peat; 50% sand and Lithovit as foliar fertilizer product.

Ersoy et al. (2016) studied the rooting of green apical cuttings of *Cotoneaster horizontalis* using IBA and air humidity. The highest root number was obtained from 4000 ppm dose application (28.90 number/cutting) and the longest root (5.39 cm) was obtained from 1000 ppm IBA hormone dose application. Also, the highest rooting area lenght was found 5000 ppm hormone dose (13.26 cm).

The selected species of the *Cotoneaster* genus as objects of the present research are promising for landscaping in our climatic conditions, therefore the features of their reproduction and cultivation require further scientific justification. Scientific advances in previous years have shown that *Cotoneaster* cuttings have good rooting ability. Our work complements these studies with documented and analyzed material that tracks the rooting percentage and development of these species under the influence of modern growth regulators and using inexpensive substrates in conditions of fine-dispersed moistening. Our research will improve propagation technology, as well as reduce production costs and ensure the planned quantity and quality of planting material.

MATERIALS AND METHODS

Studies of the influence of growth stimulants and substrates on the rooting of cuttings of *C. ascendens, C. hjelmqvistii, C. horizontalis, C. nan-shan, C. dammeri* were conducted in greenhouse, with mist irrigation in the experimental and production nursery of the National Dendrological Park "Sofiyivka" of the NAS of Ukraine.

Material for cuttings was selected from young plants whose branches were formed in the current and last year. Green cuttings were harvested from the second decade of June to the second decade of July and semi-woody *Cotoneaster* cuttings from the second decade of July to the second decade of August. We prepared the cuttings with hand pruners. We divided the cuttings into three fractions: from the apical, medial, and basal parts of the shoot. An important condition for selecting cuttings was well-developed axillary buds. The length of the cuttings ranged from 5 to 15 cm, depending on the length of the internode. Each variant of the experiment contained 25 cuttings in three replicates.The lower cut was made below the bud at an angle of 40°-50°, and the upper cut was made directly above the bud.

In order to increase the regeneration ability, the basal part of the cuttings was treated with biologically active substances (Table 1) at a room temperature of + 22°C.

The use of stimulants was carried out according to the manufacturers' instructions. The control cuttings were soaked in water.

Cuttings were planted at a depth of 1.5-5 cm, placed them in the substrate at a 15-25° slope, at a distance of 3–5 cm within row, and 7-10 cm inter-row spacing. According to recommendations Bilous (2005) relative air humidity was maintained around 85-100% using mist irrigation. By watering the cuttings for 1.5 months, the substrate humidity was maintained at 60-70% and the temperature at 20-30°C.

We used peat substrate Mixkar MKS-1 0-6 (pH 5.2-6) - substrate I; peat + sand 2:1, (pH 4.8) - substrate II; sand (pH 5.7) - substrate III. The regenerative ability and effectiveness of the studied preparations were determined by such criteria as: rooting percentage, the number of formed roots, their length, and shoot growth.

Table 1. Growth stimulants for cuttings

Stimulant name	Active ingredient	Concentration	Processing time (hours)
Heteroauxin	potassium salt of ß-indoleacetic acid (50 g/kg)	4 g/l	16
Epin +	solution of 24-epibrassinolide in alcohol (0.025 g/l)	1 ml/l	24
Kornevin	indolyl-3-butyric acid (5 g/kg)	dry powder	1
Charkor	2,6-dimethylpyridine-1-oxide and α-phenylacetic acid 8.3 g/l	1 ml/l	20

For quantitative analysis, at least 25 shoots were taken per experimental variant. Percentage of rooted plants and quantitative parameters of their development were defined within 45 days. Parameters of rooted plants, in particular the shoot growth, number of roots, and root system length were determined as the average of the measurements of 15 separate plants. Results were presented as mean value ($\bar{X}$) ± standard error (SE). The differences between means were tested with Tukey's HSD. Differences with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

Recording and comparative analysis of the results showed that the intensity of the rhizogenesis process at the initial stages depended on growth regulators and substrate. In general, the use of stimulants had a positive effect on rooting. Green cuttings of *C. dammeri* treated with a Heteroauxin solution and planted on substrate I, had the highest rooting percentage (100%), compared to the control - 72% (Figure 1, Figure 4a).

Substrate II and the stimulants Heteroauxin and Charkor, also stimulate the rooting of all green cuttings in *C. dammeri* (Figure 2).

The lowest percentage of rooting was found in green cuttings of *C. hjelmqvistii* (49.3%) using substrate II and *C. nan-shan* (50%) using substrate III without a stimulator (Figure 3 and Figure 4b, 4c).

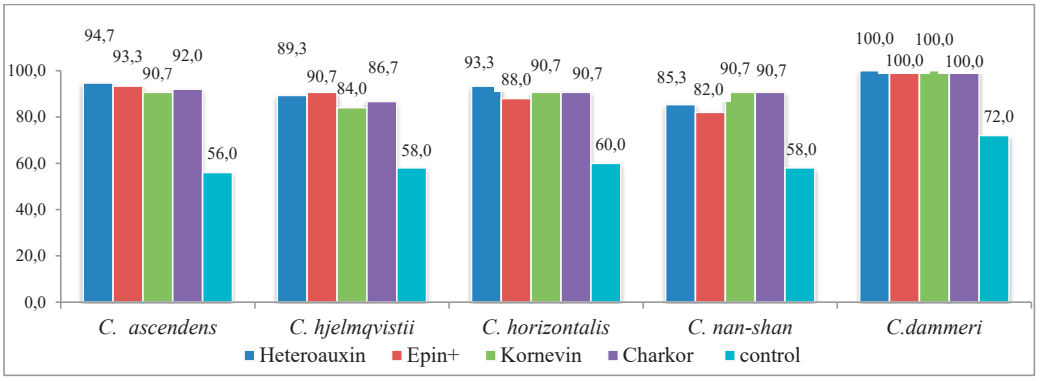


Figure 1. Effect of growth stimulant and substrate I on rooting of green cuttings (%)

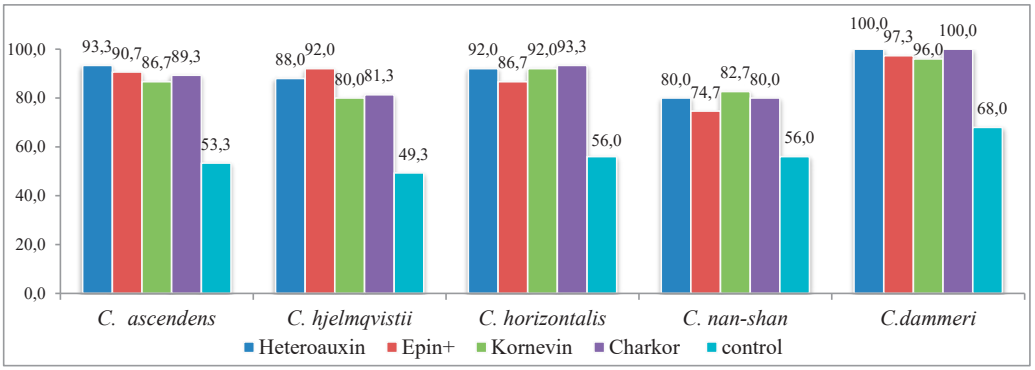


Figure 2. Effect of growth stimulant and substrate II on rooting of green cuttings (%)

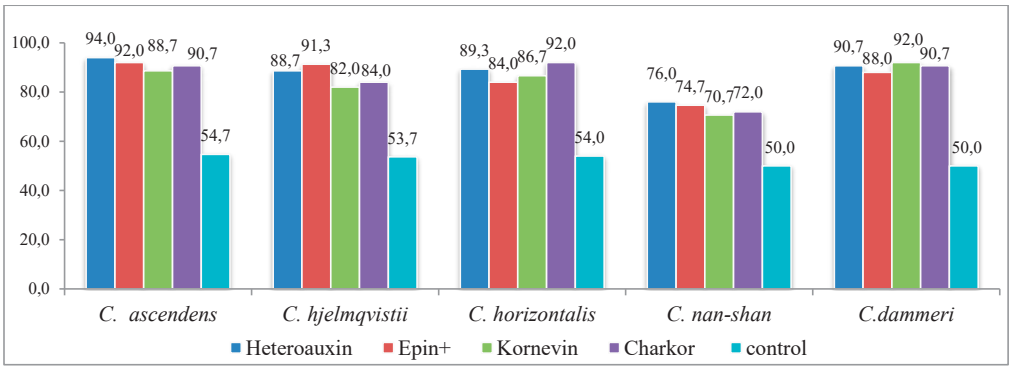


Figure 3. Effect of growth stimulant and substrate III on rooting of green cuttings (%)

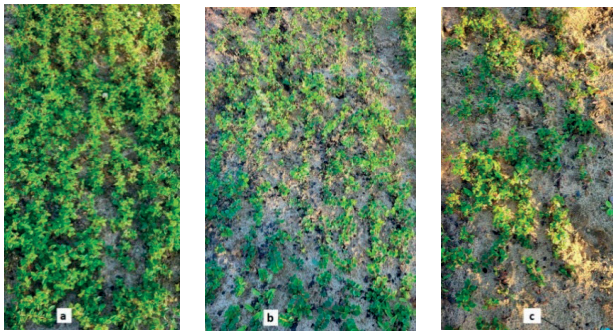


Figure 4. Rooted cuttings

a) *C. dammeri,* Heteroauxin, substrate I*;* b) *C. nan-shan,* control, substrate III*;* c) *C. hjelmqvistii,* control, substrate III

The highest rooting percentage of semi-woody cuttings of *C. ascendens, C. hjelmqvistii, C. nan-shan* and *C. dammeri* (Figure 8a) was obtained when was used Heteroauxin solution and substrate I (Figure 5). Kornevin stimulator also induced 100% rooting in both *C. hjelmqvistii* and *C. horizontalis* cuttings. Charkor stimulator and substrate I, obtained 100% rooting in *C. nan-shan* and *C. dammeri*.

The lowest rooting result (60%) was found in the control variant (*C. horizontalis, C. nan-shan*). Heteroauxin stimulator and substrate II obtained the highest percentage of rooting of *C. horizontalis* (100%) (Figure 6).

We found the lowest rooting percentage (54%) in semi-woody cuttings of *C. horizontalis* (control variant) in substrate III (Figure 7).

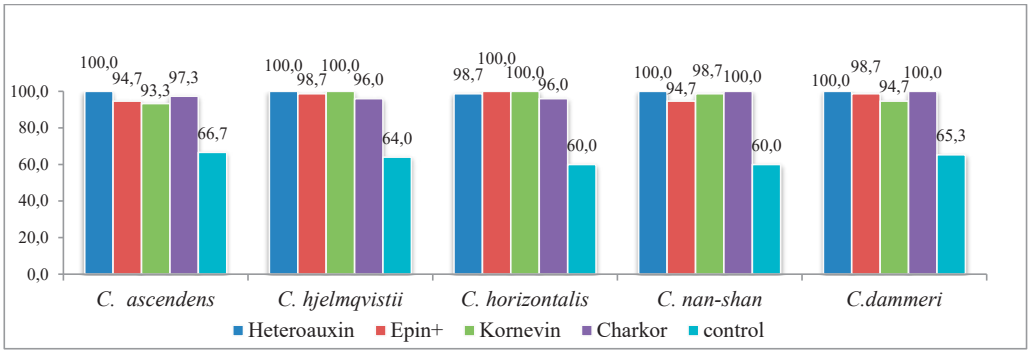


Figure 5. Effect of growth stimulant and substrate I on rooting of semi-woody cuttings (%)

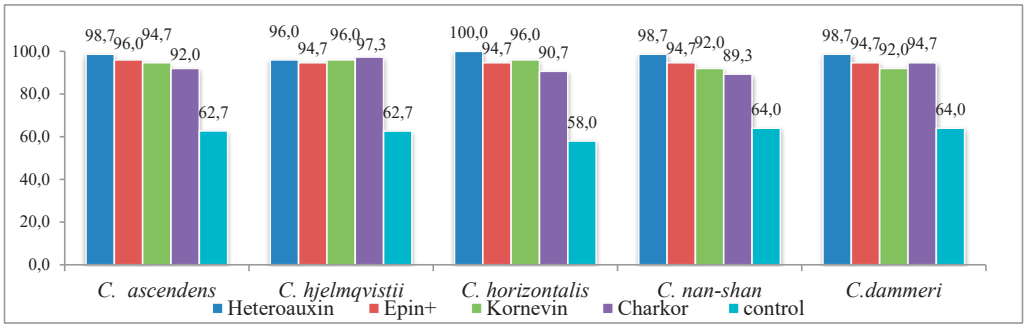


Figure 6. Effect of growth stimulant and substrate II on rooting of semi-woody cuttings (%)

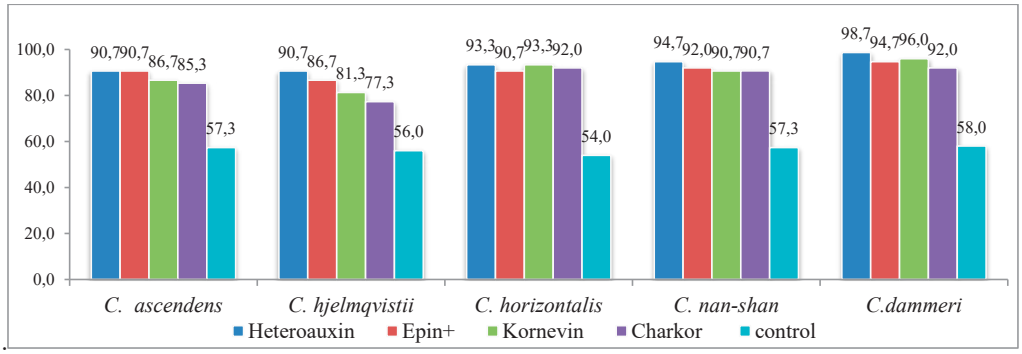


Figure 7. The effect of growth stimulant and substrate III on rooting of semi-woody cuttings (%)

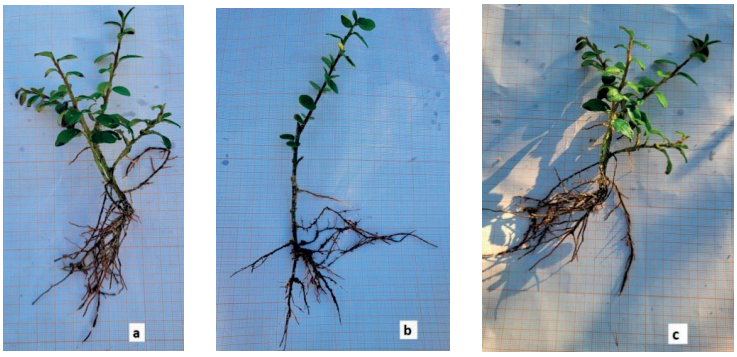


Figure 8. Rooting of green cuttings of plants of the genus *Cotoneaster* 45 days after planting in substrate I
a) *C. dammeri,* Heteroauxin; b) *C. ascendens,* Epin+; c) *C. nan-shan,* Charkor

The use of stimulants and various substrates has a positive effect on the percentage of rooting of cuttings, as well as on the growth and development of the root system and the above-ground part of the cuttings (Tables 2, 3, Figure 8).

Shoot growth, number of roots, and length of the root system were not significantly different when using the stimulants Heteroauxin, Epin+, Kornevin and Charkor in green cuttings of *Cotoneaster* species (Table 2). With the use of stimulants, the height of the above-ground part of rooted green cuttings exceeded the control variant by 1 cm. The number of roots per plant in the control variant was on average 2 fewer, and their length was 1.9 cm less. It was found that the use of substrate I was more effective (Table 2, Figure 8, Figure 9).

Biometric indicators such as shoot growth, number of roots, and length of the root system of rooted semi-woody cuttings almost did not differ (Table 3). The growth of the above-ground part of the cuttings using stimulants exceeded the control by 0.6 cm, the number of roots exceeded by 1.4 pcs and the length of the root system was 1.6 cm longer. The best result was obtained when using substrate I (Table 3, Figure 9a).

Table 2. Characteristics of green cuttings of plants of the genus *Cotoneaster* 45 days after planting (n=15, X̄±SE)

Species	Shoot height, cm			Number of roots, pcs.			Root system length, cm		
	Substrate, №			Substrate, №			Substrate, №		
	I	II	III	I	II	III	I	II	III
With stimulants									
C. ascendens	2.3±0.10^{a}	1.8 ±0.11^{a}	1.1±0.08^{a}	4.0±0.28^{a}	4.8 ± 0.26^{a}	2.8±0.24^{a}	7.4±0.42^{a}	5.9 ± 0.35^{a}	4.6±0.21^{a}
C. hjelmqvistii	2.8±0.11^{b}	2.4±0.12^{b}	1.1±0.09^{ac}	3.1±0.31^{b}	2.9±0.24^{b}	2.1±0.18^{b}	5.4±0.28^{b}	4.9±0.38^{b}	4.5±0.37^{ac}
C. horizontalis	3.0±0.10^{c}	2.8±0.12^{c}	1.2±0.08^{ad}	4.8±0.28^{c}	4.7±0.25^{a}	3.0±0.26^{a}	5.5±0.40^{b}	5.1±0.33^{b}	4.9±0.28^{ad}
C. nan-shan	2.2±0.13^{a}	1.8±0.17^{a}	1.8±0.14^{b}	3.9±0.25^{a}	3.3±0.25^{c}	2.2±0.24^{b}	5.6±0.26^{b}	5.0±0.32^{b}	4.6±0.34^{ac}
C.dammeri	3.2±0.18^{d}	2.7±0.13^{c}	1.2±0.10^{cd}	5.8±0.30^{d}	4.0±0.31^{d}	3.1±0.27^{a}	7.3±0.37^{a}	6.1±0.33^{a}	5.2±0.30^{bd}
X̄	2.7	2.3	1.3	4.3	3.9	2.6	6.4	5.5	4.7
Control									
C. ascendens	1.7±0.13^{a}	1.5 ± 0.14^{a}	1.0±0.07^{ac}	3.3±0.23^{a}	3.8 ± 0.33^{a}	2.3±0.18^{a}	5.4±0.28^{a}	4.6 ± 0.30^{a}	3.7±0.20^{a}
C. hjelmqvistii	1.6±0.13^{ab}	1.7±0.18^{bc}	1.0±0.08^{a}	2.7±0.23^{b}	2.3±0.23^{b}	1.9±0.18^{b}	4.1±0.23^{b}	3.8±0.19^{b}	3.7±0.24^{a}
C. horizontalis	1.5±0.13^{bc}	1.6±0.14^{ac}	1.0±0.07^{ac}	3.5±0.31^{a}	3.5±0.27^{ac}	2.3±0.25^{a}	4.0±0.22^{b}	3.0±0.20^{c}	3.1±0.20^{b}
C. nan-shan	1.4±0.14^{cc}	1.2±0.09^{c}	1.2±0.12^{b}	3.1±0.29^{a}	2.5±0.27^{b}	1.9±0.24^{b}	3.8±0.21^{b}	3.8±0.20^{b}	3.5±0.23^{a}
C.dammeri	2.2±0.19^{d}	2.0±0.17^{d}	1.1±0.10^{c}	4.1±0.35^{c}	3.3±0.28^{c}	2.4±0.27^{a}	5.0±0.26^{c}	4.5±0.32^{a}	4.3±0.20^{c}
X̄	1.7	1.6	1.1	3.3	3.1	2.2	4.5	3.9	3.7

Note: the different letters indicate statistically significant differences in the means of the compared pair according to the Tukey criterion

Table 3. Characteristics of semi-woody cuttings of plants of the genus *Cotoneaster* 45 days after planting (n=15, X̄±SE)

Species	Shoot height, cm			Number of roots, pcs.			Root system length, cm		
	Substrate, №			Substrate, №			Substrate, №		
	I	II	III	I	II	III	I	II	III
With stimulants									
C. ascendens	2.0±0.14^{a}	1.7 ± 0.11^{a}	0.9±0.07^{a}	4.9±0.34^{a}	4.6 ± 0.27^{a}	2.6±0.19^{a}	6.3±0.44^{a}	5.2 ± 0.35^{a}	4.7±0.40^{a}
C. hjelmqvistii	2.2±0.18^{b}	2.3±0.15^{b}	1.2±0.16^{b}	4.3±0.40^{b}	3.2±0.28^{b}	2.4±0.19^{a}	4.8±0.38^{b}	4.0±0.28^{b}	5.5±0.44^{b}
C. horizontalis	2.9±0.10^{c}	2.2±0.21^{b}	0.9±0.07^{ac}	4.3±0.34^{b}	3.7±0.35^{c}	2.9±0.21^{b}	5.5±0.44^{b}	5.1±0.32^{ab}	4.6±0.35^{a}
C. nan-shan	1.7±0.19^{d}	1.4±0.15^{c}	1.0±0.11^{c}	4.1±0.27^{b}	3.2±0.26^{b}	2.4±0.21^{a}	5.6±0.39^{b}	4.9±0.34^{b}	4.7±0.30^{a}
C.dammeri	2.6±0.18^{c}	2.1±0.18^{b}	1.1±0.09^{bc}	5.3±0.33^{c}	4.0±0.32^{c}	2.5±0.24^{a}	6.8±0.41^{a}	5.3±0.31^{a}	4.6±0.26^{a}
X̄	2.3	1.9	1.0	4.6	3.7	2.6	5.9	5.1	4.5
Control									
C. ascendens	1.4±0.14^{a}	1.3 ±0.12^{a}	0.7±0.09^{a}	3.0±0.31^{ac}	2.7 ± 0.30^{a}	2.1±0.23^{a}	4.7±0.22^{a}	4.1 ± 0.20^{a}	3.8±0.23^{a}
C. hjelmqvistii	1.4±0.13^{a}	1.4±0.13^{b}	1.0±0.10^{b}	2.9±0.26^{a}	2.3±0.27^{b}	1.9±0.21^{a}	4.0±0.24^{b}	4.0±0.32^{ad}	3.2±0.17^{b}
C. horizontalis	2.1±0.13^{b}	1.8±0.19^{c}	0.7±0.07^{ad}	2.7±0.25^{ad}	2.7±0.30^{a}	2.3±0.21^{b}	3.8±0.23^{b}	3.8±0.18^{bd}	3.±0.22^{b}
C. nan-shan	1.4±0.17^{a}	1.2±0.09^{a}	0.8±0.12^{cd}	3.1±0.25^{ac}	2.4±0.19^{b}	1.9±0.18^{a}	3.8±0.22^{b}	3.7±0.16^{b}	3.2±0.16^{b}
C.dammeri	1.8±0.09^{c}	1.6±0.14^{c}	0.9±0.08^{c}	3.6±0.27^{b}	2.9±0.27^{a}	1.7±0.15^{c}	5.1±0.20^{c}	4.2±0.13^{a}	3.5±0.16^{c}
X̄	1.6	1.5	0.8	3.1	2.6	2.0	4.3	4.0	3.4

Note: the different letters indicate statistically significant differences in the means of the compared pair according to the Tukey criterion



Figure 9. Rooted semi-woody cuttings of plants of the genus *Cotoneaster* (45 days)
a) *C. horizontalis* Heteroauxin, substrate I; b) *C. hjelmqvistii* control, substrate II; c) *C. ascendens* control, substrate III

A comparative analysis of the results obtained in our studies with the conclusions of other authors showed that the use of stimulants had a positive effect on the rooting of cuttings. Kovalchuk et al. (2021) using the Heteroauxin stimulator and substrate II, reported high rooting rates of 94% of green cuttings of *Buxus sempervirens* L., which exceeded the control by 18%.

In our experiments, all green cuttings on Substrate II + Heteroauxin, have rooted in the species *C. dammeri*. Quite high rooting results were also obtained for *C. ascendens* - 93.3%, *C. horizontalis* - 92%, *C. hjelmqvistii* - 88%, but this combination was less effective in promoting rooting of *C. nan-shan* - 80%.

Rooting green cuttings of *Buxus sempervirens* L. according to the scheme Substrate II + Kornevin, Kovalchuk et al., (2021) achieved an efficiency level of 93%, which exceeded the control by 16%. In our studies, the same scheme (Substrate II + Kornevin) was most effective for green cuttings of *C. dammeri* (96%). When rooting *C. horizontalis*, the use of stimulants such as Kornevin or Heteroauxin leads to the same rooting result (92%). However, scheme Substrate II + Kornevin recorded slightly lower results at *C. ascendens* - 86.7%, *C. nan-shan* - 82.7% and *C. hjelmqvistii* - 80%.

Green cuttings of *Buxus sempervirens* on Epin+ and Substrate II, formed roots in 93% of the cuttings, as in the variant with the Kornevin stimulator, which exceeded the control by 16% (Kovalchuk et al., 2021). Using the same scheme, we obtained a fairly high result for *C. dammeri* - 97.3%, *C. hjelmqvistii* - 92%, *C. ascendens* - 90.7%, and for *C. horizontalis* and *C. nan-shan*, was less effective - 86.7 and 74.7%, respectively.

In the scheme Substrate II and control the rooting results were quite low: *C. ascendens* - 53.3%, *C. hjelmqvistii* - 49.3%, *C. horizontalis* - 56.7%, *C. nan-shan* - 56%, *C. dammeri* - 68%. In general, when using stimulants, the percentage of rooting of green cuttings of species of the genus *Cotoneaster* was higher by 18.7-42.7%.

Comparing the rooting rate of green cuttings of the species *C. ascendens, C. horizontalis, C. nan-shan* with the results of the experiments of Grevtsova & Kazanskaya (1997), it was found that in our studies the rooting rate is higher. For example, the maximum yield of rooted green cuttings of *C. nan-shan* was 90% when using indoleacetic acid and leafy soil as a substrate. In our study, the maximum yield of rooted green cuttings of *C. nan-shan* was 90.7%, using Kornevin and Charkor and substrate I (peat Mixkar MKS-1 0-6) (Figure 1). The maximum yield of rooted green cuttings of *C. ascendens* was 85% when using indolylbutyric acid and the substrate leaf soil, and in our studies 94.7% when using Heteroauxin and the substrate I. The maximum yield of rooted green cuttings of *C. horizontalis* was 83% when using indolylbutyric acid and the substrate leaf soil, and in our studies 93.3% when using Heteroauxin and the substrate I. The maximum yield of rooted green cuttings of *C. nan-shan, C. ascendens, C. horizontalis* in the control variant (talc + sand) was 48, 44, 41%, respectively, and in our studies 58, 56, 60%, respectively, when using substrate I.

Comparing the rooting efficiency of semi-woody cuttings of the species *C. ascendens, C. horizontalis, C. nan-shan* with the results of experiments in our studies, it was found that in our studies, rooting efficiency is also higher. The maximum yield of rooted semi-woody cuttings of *C. ascendens* was 92% when using indolylbutyric acid and the substrate leaf soil, and in our studies 100% when using Heteroauxin and substrate I (Figure 3). The maximum yield of rooted semi-woody cuttings of *C. nan-shan* was 79% when using indoleacetic acid and leaf soil substrate, and in our studies 100% when using Heteroauxin and Charkor and substrate I. acid and the substrate leaf soil, and in our studies 100% when using Heteroauxin and Charkor and the substrate I. The maximum yield of rooted semi-woody cuttings of *C. ascendens, C. nan-shan,* and *C. horizontalis* in the control variant (talc + sand) was 63, 40, and 25%, respectively, and in our studies it was 66.7% when using (substrate I), 64% (substrate II peat + sand 2:1), and 60% (substrate I), respectively.

A study of the effect of stimulants on the rooting of green and semi-woody cuttings was conducted by Kopylova (2021) on the species *Pyracantha coccinea, P. crenatoserrata*, and *P.* x 'Orange Charmer'. Growth stimulants accelerated and improved the rooting of *Pyracantha* cuttings. The best results (100%) in rooting green pyracantha cuttings were obtained when using Heteroauxin and in KTS substrate (2:1:1) and peat tablets. The onset and mass rooting occurred 6-8 days earlier than in the control, the difference in the average root length was from 6.2±1.24 to 9.64±4.5 cm, the number of roots from 4.4±1.88 to 6.2±1.36 pcs., the average shoot growth from 1.8±0.49 to 2.16±0.77 cm three months after cutting.

In our studies on rooting with green cuttings, the greatest shoot growth of 3.2±0.18 cm was found in plants of the species *C. dammeri*, and in plants of the species *C. ascendens* the greatest root system length was 7.4±0.42 cm in the variant using growth stimulants and in substrate I. When rooting with semi-woody cuttings, the greatest shoot growth of 2.9±0.10 cm was found in plants of the species *C. horizontalis*, and in plants of the species *C. dammeri* the greatest root system length was 6.8±0.41 cm in the variant using substrate I. We obtained the best biometric indicators when rooting green and semi-woody cuttings of plants of the genus *Cotoneaster* using stimulants and peat in the substrate.

CONCLUSIONS

It has been proven that in order to root a certain species of the *Cotoneaster* genus, it is necessary to use a specific type of growth stimulant and substrate. For semi-woody cuttings the best stimulator was Heteroauxin for *C. ascendens*, Heteroauxin and Kornevin for *C. hjelmqvistii* Kornevin and Epin+ for *C. horizontalis*, Heteroauxin and Charkor for *C. nan-shan*, Heteroauxin and Charkor for *C. dammeri*.

For green cuttings of *C. dammeri* Kornevin stimulator, promoted rooting at a level of 92.0 to 100%, Heteroauxin and Charkor - from 90.7 to 100%; and the Epin+ stimulator - from 88.0 to 100%.

An important factor in the technology of production of standard planting material is the substrate. The best substrates for rooting the studied species of the genus *Cotoneaster* were Mixkar MKS-1 0-6 pH 5.2-6 and peat + sand 2:1, pH (4.8).

When rooted using stimulants, cuttings formed greater shoot growth and a deeper root system. Also, shoots growth, of green cuttings was better. The number of roots under the influence of stimulants increased to a level of 2.6 to 4.3 pcs., and the length of the root system from 4.7 to 6.4 cm.

When stimulants were used on semi-woody cuttings, the growth of shoots ranged from 1 to 2.3 cm, and without the stimulant from 0.8 to 1.6 cm. The number of roots increased to a level of 2.6 to 4.6 pieces, and the length of the root system of 4.5 to 5.9 cm.

Therefore, we recommend these stimulants and substrates for mass propagation.

The practical significance of the research results for industrial plant nurseries lies in the possibility of improving the technology of propagation of *Cotoneaster* species through cost-effective growth regulators. Establishing sustainable agronomic practices is an important step in introducing plants of the *Cotoneaster* genus into ornamental gardening, which create long-lasting and decorative plantings

throughout the growing season, while requiring minimal care.

Our research may be useful for future studies on how growth promoters affect root or shoot formation. These data are important for basic research in plant physiology and may also form the basis for further research in plant breeding.

The results of our work can be used to improve laboratory methods for studying the effects of growth stimulants on other plant species, and will also help to evaluate the effectiveness of different stimulants and substrates in comparison.

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IRIS SUAVEOLENS BOIS. & REUT. - MORPHOLOGICAL FEATURES FOR ATTRACTING AND SUPPORTING POLLINATORS

Elena MELUȚ, Elena SĂVULESCU, Vasilica LUCHIAN, Mihaela Ioana GEORGESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaelaioanageorgescu@yahoo.com

Abstract

In this study, a Romanian dwarf specie of the genus Iris (Iris suaveolens Bois. and Reut.) is analysed regarding the floral morphology and pollinators relationship. This specie is known as very important ornamental plants due to their flowers and dwarf habitus. The Iris flower is considered a floral complex or a pseudo-inflorescence because of their three pollination canals which serve like an independently zygomorphic flower. The floral elements were investigated with regarding the dimensions and pollination adaptation through a population observation and microscopical analysis of the flower elements. The three standards are longer and wider than falls. The falls are reflexed and covered with a band of coloured hairs (beards). These floral complexes are very attractive not only for insects but also for their aesthetic potential The whole floral complex is as tall as the scape and is highly specialised in attracting insects in several ways, by providing food or protection. Studied and using wild Iris species in gardens is beneficial choice for both species population and also for pollinators support.

Key words*: flowers, Iris, morphology, ornamental, pollinators.*

INTRODUCTION

The Irises are flowering plants with an important symbolistic, artistic and scientific history, very deep related with the entire life and spirituality. In the last centuries the irises were fascinating also the scientific society, being intense studied, classified and being included in important ornamental plant breeding programs.

The Irises are well known as medicinal and ornamental garden plant in the Romanian area for centuries (Butură, 1979; Ardelean & Mohan, 2008).

Iris L. genus is largest monocot genus in the *Iridaceae* family, that gathers together about 300 species. This large genus provide species which are important members in the wild flora of many countries, including the Romanian one (Goldblatt et al., 2008).

Along the evolution, the majority of the Iris species have developed highly specialised strategies for entomophilous pollination. The reproductive self-incompatibility of the Iris species facilitated the development of a mutually beneficial relationship with the pollination insects.

The upper tepals calls standards protect the reproductive structures and the lower tepals called falls provide a landing support for insects (Robu, 2005; Uno, 1982).

Latest studies from worldwide shows us that the useful entomofauna and particularly the pollination insects, cross a difficult period due to different types of pollution (www. environment. ec. europa. eu).

In this context, study the floral structures and morphology that attract insects, can provide important information about the plant species that can help to conserve the wildlife in its large complexity and to support the concept of resilient gardens with a specific of each natural areas.

In the last years the studies highlighted the importance of Romanian wild Irises, in the context of decreasing the natural Iris population across Europe and the need of increasing the conservation efforts to protect our local species (Crișan et al., 2017).

The conservation approaches includes studied the local species, gather information about their biology and the benefits of using them in the gardens for both ornamental or ecological traits.

The aim of this article is to provide information about an important wild Iris specie in Romanian flora and to highlight its morphological and ecological traits that upgrade it at the rang of both ornamental and wildlife supportive specie.

MATERIALS AND METHODS

Iris suaveolens Bois. & Reut. is a dwarf perennial rizomatous specie, growing wild in Balkans area, included Romania (Dobrogea, South-Est region), on steppe areas or calcareous substrates, being a xerophyte specie.
The local authors and not only, described its flower with purple or yellow delicate tepals, growing in clusters. It show glaucous light green leaves, ensiform and ribbed by the protuberant parallel venations (Colasante, 2018).
It blooms in middle spring (April-May) and is a very important specie regarding the Romanian biodiversity (Prodan & Nyarady, 1966; Matew, 1981).
Due to its complexity, some authors consider the Iris flower as a pseudo inflorescence, consisting in three pollination units, called meranthia, each serving like an independently labiate or zygomorphic flower (Uno, 1982; Guo, 2015).
A population of *Iris suaveolens* Bois. & Reut. was located in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, originated from North Dobrogea (Tulcea County) and was studied during a couple of vegetation seasons.
The Botanical Garden of the University is located in Bucharest, in South region of Romania, in the oak - forest zone, with temperate climate with excessive tendencies for the last decade.
The soil in city area is developed under the action of natural and artificial factors, getting loamy and alluvial due to humus erosion by the surface water (www.pmb.ro).
For understanding the way this specie developed and supports the wild life regarding the pollination insects, there were conducted a series of morphological observations, regarding the dimension, shape, colour of the floral elements through biometric determinations and microscopical investigations regarding the flower elements adaptation to the entomophilous pollination, the way that the floral structure attract the insects and provide them several rewards.
The *Iris suaveolens* population was studied regarding the anthesis period, to understand how long the insects can benefit from this flower support.
The morphological features were studied at the full anthesis and illustrated in the article in the detailed photos.
The detailed images were obtained by replacing the background and separating each elements for highlighting the main features, using an editing program.
The microscopical analysis of bearded tepals (falls) was made by investigating the epidermal structure adapted for attracting insects (stripes and hair) with electronic microscopy methods.

RESULTS AND DISCUSSIONS

Iris suaveolens Boiss and Reut is a dwarf specie, is included in the Miniature Dwarf Bearded category for plant breeding works.
The entire plant height in the studied population, varied between 11-14 cm, with an average by 12.5 cm (Table 1).

Table 1. External morphology analysis of *Iris suaveolens* Bois. & Reut.

Analyzed organs	Average of length (cm)	Average of width (cm)
Plant height	12.5	-
Perigon	6.5	4.5
Perigon tube	6.5	0.5
Standards	4.5	2.7
Falls	4.5	1.7
Fillaments	1.8-2	-
Anthers	1.5	-
Ovary	1.2	0.7
Stile	1.7	1.3
Capsule	4.0	1.8

From this entire height, only the flower riches, an average about 6.5 cm long (Table 1).
As many authors have written, the iris flower can be described as a floral complex, consisting different structures, which were studied in the local population regarding their dimensions. The three standards (upper tepals) can achieve an average 4.5 cm in length and 2.7 cm in width. At the flowering time, the standards are united together at the top, creating a dome like flower architecture.

The purple standards have visible venation, wavy and turned outwards margins (Table 1).



Figure 1. *I. suaveolens* flower (St- standards)
(Source: original photo)

The lower tepals (falls), purple in colour as well, are curved down (reflected), oblong in shape and riches an average length of 4.5 cm and width of 1.7 cm. The three falls act in the flower like a landing platform for insects.
Together with the dome like standards, it can provide home and shelter to the entomophilous visitors. The width of falls is enough to support the insect. Besides that, the falls are thicker than standards, character that can facilitate supporting weight of the insect.



Figure 2. *I. suaveolens* falls
(Source: original photo)

Every fall, in order to contribute in attracting pollinators, developed a visible network of dark purple to brown strips and a band of white hairs (Figure 2.b).
At the basis of the falls, the colour is getting progressively light green and the hairs colour, too.
The fall is developed directly from the perigon tube, which is also light green, almost circular and every fall is continuously growing from each tube canal, breakaway from the trilocular ovary.

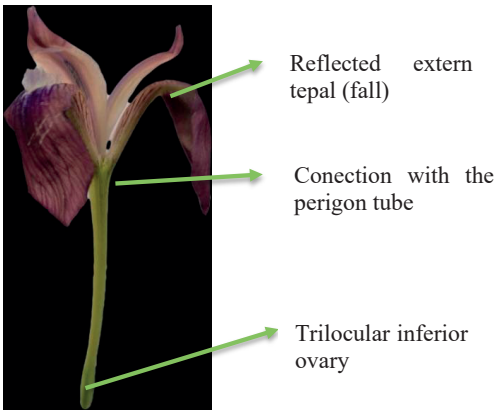


Figure 3. *I. suaveolens* perigon tube and falls
(Source: original photo)

The falls seems to provide the highest specialization to the entomophilous pollination. Their auxiliar structures and chromatic act together like signals for insects (Robu, 2005).
The microscopical analysis of the external tepals at the hair band, shows the pluricellular, turgid hairs (Figure 4-a, c) and the dark purple stripes.

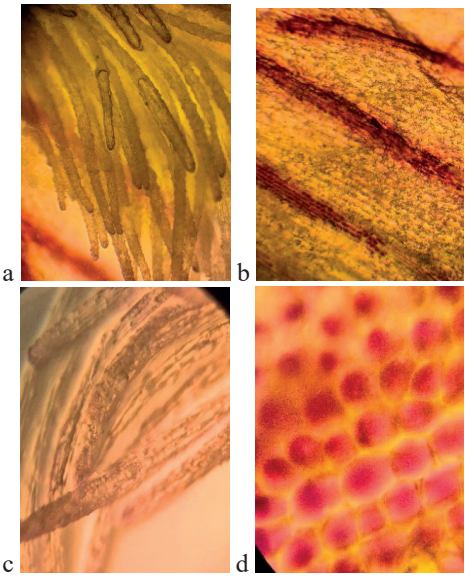


Figure 4. Microscopical view of falls hairs (a,c) and anthocyanin stripes (b,c)
(Source: original photo)

The Figure 3, illustrates microscopic images of the bearded hairs and the stripes nearby. It can be observed that the stripes are compound by several (4-8) rows of anthocyanin content cells, in the tepals parenchyma.
The colored strips serve like a visual signal for insects and the pluricellular hair band could provide the pollen dispersal, a tactile stimulation or helping the smaller insects to land or stay on the fall surface.
The stile dimensions observations indicate an average about 1.5 cm in length and 1.3 cm in width (Table 1).
The stile and stigma are described having petaloid aspect, oblong shape and entire, concave aspect and thicker on a longitudinal darker strip, looking like a protuberant keel (Săvulescu et al., 1966).
Along the stile, in the middle region the structure becomes thicker and darker in color, this is a canal that make the connection between the stigma and the perigon tube (Figure 5).

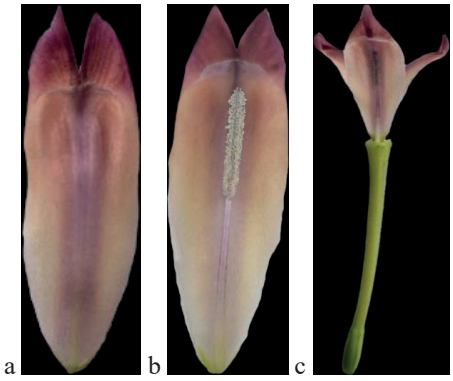


Figure 5. Stile and stigma (a, b); connection with the perigon tube (c)
(Source: original photo)

The stigma is a bilobed structure, each lobe has triangular shape, the color is purple, like the tepals, concave but in the opposite direction regarding the stile. The two lobes of the stigma are apart one of the other by a large angle and the margins are serrates (Figure 5).
Underneath each one of the three stigma structures of the flowers, is located one stamen. This is shorter and completely covered by the stile, intimately following the concave line but not reaching the upper part of the stigma, this fact could represent one of the arguments of the self-incompatibility and the need for a pollination agent (Figure 5b).
As previously mentioned and as many authors highlighted along the studies, the flower in Iris genus consist in a three structure unit, which is replicated three times.
Every unit is composed by a group: stigma, stamen and fall, protected by the dome like standards (Figure 6).
This unit act in the pollination process like an independent labiate flower. Authors named these pollination units and consider the hole flower being a pseudo inflorescence (Goldblatt et al.,1998; Guo, 2015).
In the current study, every pollination unit of the *I. suaveolens* flower was disassembled in component parts (Figure 6a), to understand the each one role in the pollination process.

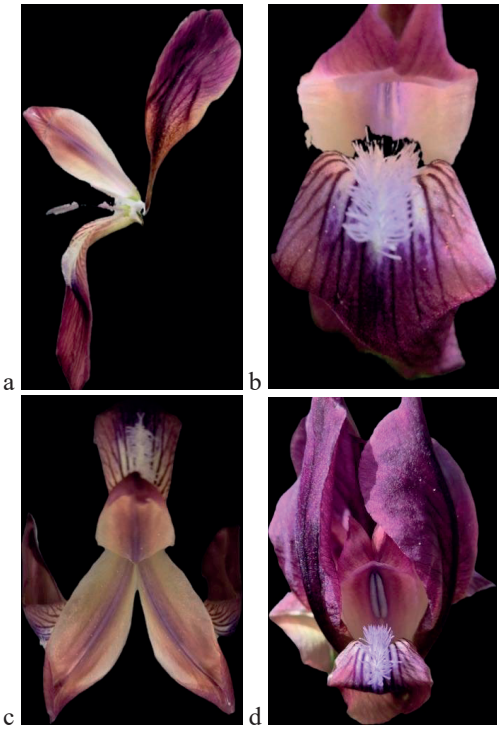


Figure 6. *I. suaveolens* flower (a-pollination unit disassembled, b-pollination unit entire, c-the three pollination units, d-entire flower, pollination unit foreground) (Source: original photo)

In the Figure 6(a) the pollination unit is observed disassembled, compound by the fall, which support the stationary insects, the reproductive organs, stamen and stile upwards the fall and a

standard from the three ones, that protect the reproductive organs system.
The pollination insects can be faithful to one pollination unit or can visit any of the unit of ine flower (Uno, 1982).
The way that the stigma is curved over the fall, facilitates the direct contact with the dorsal part of the insect and provide the existing pollen to get in the pollenic tube towards the ovary.
Because the nectar is not easy accessible to the insect, in many species the nectary structure are located to the base of perigon tube, these species can be pollinated by having a deep access in the flower unit, to provide the anther to touch the dorsal part of the insect body.
In the Figure 6d, can be easily observe the position of the anther under the stigma, located just above the de hair band of the fall.
As usual in nature, the mutual beneficial relationship between Iris flowers and pollination insects, both parties are advantaged.
If the flower specie reproductive success depends on the insect visitors, the insect land on the flower structure for a reward.
Studies shows that in the genus Iris the pollinator support can vary from the nectar, pollen or even shelter or home for the small ones (Dabrowska et al., 2019).
Regarding this information, the pollination unit described before and the protective dome like standards, look like having the architecture of a shelter/home for some visitors (Figure 6d).
The flowering period or the anthesis lasting was registered in the two vegetation periods regarding the number of days from the flower buds stage to the end of flowering (fecundation phenophase).

Table 2. Phenological phases of flowering at *I. suaveolens* and duration along the spring time

Flower phenophase	First season (2024)			Second season (2025)		
	April (day)		May (day)	April (day)		May (day)
	1-14	15-30	1-15	1-14	15-30	1-15
Flower buds						
Open flower						
End of flowering						

In Table 2, can be observed that in the first season, the flower bud occurrence was observed in the first two weeks of April, comparing with the second study season, when this phenophase was observed in the middle to the second part of April.
The full flowering open occurred, both seasons in the second half of April, the full flowering period analyzed at the entire population last about 14 days and analyzed at a single flower it last about 5 days.
The third stage of the anthesis, end of flowering, in the first study season occurred in the first half of May and in the second season was longer, starting earlier, by the end of April.
In 2024 spring season, when the whether conditions were less rainy and warmer the I. suaveolens population achieved a longer flowering period, compared with the 2025 spring season, which is more rainy and cold, so the flowering period last less. This flowering conditions highlight the xerophytic character of the *I. suaveolens*.
The anthesis period description show that in warm springs, an *I. suaveolens* population can provide shelter and food to pollination insects during almost two weeks, by periodic flowering.

CONCLUSIONS

Iris suaveolens Boiss and Reut is a dwarf specie and the entire plant height in the studied population, varied between 11-14 cm, with an average by 12,5 cm.
As many authors have written, the iris flower can be described as a floral complex. At the flowering time, the purple standards can achieve an average 4,5 cm in length and 2,7 cm in width, are united together at the top, creating a dome like flower architecture.
The purple falls are curved down (reflected), oblong in shape and riches an average length of 4.5 cm and width of 1.7 cm. The three falls act in the flower like a landing platform for insects. Every fall, in order to contribute in attracting pollinators, developed a visible network of dark purple to brown strips and a band of white pluricellular hairs, being highly specialised in entomophilous pollination.
The stile and stigma are described having petaloid aspect, oblong shape and entire, concave aspect and thicker on a longitudinal

darker strip, looking like a protuberant keel, that entirely cover the stamen.

The pollination units are composed: stigma, stamen and fall, protected by the dome like standards, which can attract the insects and provide food and protection.

This subject needs further studies in the structure and ultrastructure of the Iris tepals, to provide a complete understanding of the relationship and behaviour of the insects visitors in the Iris floral complex.

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BIOECOLOGICAL FEATURES OF SEVERAL NEW TAXA OF *CORNUS ALBA* L. GROWN AT THE "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE)

Ion ROȘCA, Elisaveta ONICA, Alina CUTCOVSCHI-MUȘTUC, Maria TABĂRA, Natalia ONICA

"Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University, Chișinău, Republic of Moldova
Corresponding author email: amustuc@gmail.com

Abstract

The article includes the results of a research on the bioecological features of some new taxa from the collection of the genus Cornus L. of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The study includes the taxa of Cornus alba: 'Elegantisima', 'Aurea', 'Cream Cracker' PBR, 'Sibirica'. The studied taxa are known as dogwoods, having high decorative value due to their habit, crown, color of vines, leaves, fruits, long duration of flowering and fructification, all of which are determining criteria in landscape art. They are resistant to drought, frost, pollutant substances, do not require special care, only shaping cuts and compliance with the recommended technology throughout the growing season. They are used in landscaping particularly in the foreground, in small groups, in mixtures at the edges of tree plantations and hedges of different heights and colors.

Key words*: biomorphology, Cornus alba* L.*, development, growth, ornamental parameters, varieties.*

INTRODUCTION

The rapid urban development makes it necessary to provide green spaces with an assortment of plants with of high decorative value, in order to provide a microclimate that would benefit the well-being of city residents and to create favorable conditions to hold social activities. Raising living standards, including an improved urban landscape, must become a priority of modern society, and harmonizing artificial landscapes with natural ones can be successfully achieved by vegetation.

The lush appearance of the green spaces, as well as their volumetric spatial view and their coloristic expressiveness, are due to the vitalizing aesthetic composition of the selected plant species, as well as their successful association. Even if the proportion of non-native plants in the plant compositions of green spaces is significantly lower as compared with the native ones, the choice and association of taxa with high indices of decorativeness, according to their habit, crown shape, color of shoots, leaves, fruits, long duration of flowering and fruiting, are determining criteria for the successful creation of landscapes of high aesthetic value. From the multitude of woody species, taxa of the genus *Cornus* L. can be recommended for this purpose due to their important decorative indices, especially during winter against the background of snow. The family *Cornaceae* Bercht. & J. Presl includes 10 genera with about 100 species, distributed in the temperate and sub-tropical zones (Derevia i custarnichi SSSR, 1960).

The genus *Cornus* L. includes about 70 species and varieties widespread in temperate regions (http://www.theplantlist.org). In Europe, 6 species and multiple forms of high ornamental value are widespread (Iliescu, 2002) In the Republic of Moldova, 3 species (*Cornus alba* L., *Cornus mas* L., *Cornus sanguinea* L.) and diverse varieties occur (Palancean et Comanici, 2009).

The goal of this research included the study of the bioecological features of 4 new taxa of *Cornus alba* L., (*Cornus alba* 'Elegantisima', *Cornus alba* 'Aurea', *Cornus alba* 'Sibirica', *Cornus alba* 'Cream Craker' PBR) in the 5th growing season, under the conditions of the "Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University.

MATERIALS AND METHODS

The investigations were conducted in 2019-2024 as part of the project 20.80009.7007.19 (2020-2023) "The introduction and development of technologies for propagation and cultivation of new species of woody plants by conventional techniques and tissue culture" and continued within the subprogram 010101 "Research and *ex situ* and *in situ* conservation of plant diversity in the Republic of Moldova" in the plant nursery of the Dendrology Laboratory. During the study period, climatic conditions were favorable for plant growth. The highest temperatures were in 2020 $+38^0$ C, in other years the temperatures ranged on average from 25^0C to 28.5^0C. According to the data, there was a deficit of precipitation in both the winter and summer seasons, which led to the need for irrigation of plants. As research subjects served 4 taxa of *Cornus* L., (*Cornus alba* 'Elegantisima', *Cornus alba* 'Aurea', *Cornus alba* 'Sibirica', *Cornus alba* 'Cream Craker' PBR) at the end of the 5th growing season, which grow and develop in the collection of NBGI. The morphological parameters were determined for 3 specimens of each taxon, as well as for 100 flowers, leaves and shoots after regular annual pruning in spring. Phenological observations were made according to the method developed at the Moscow Botanical Garden (Metodica fenologhiceschih nabliodenii v botaniceschih cadah SSSR, 1979) and perfected by Dr. hab. A. Palancean (Palancean and Comanici, 2009).

RESULTS AND DISCUSSIONS

The contribution of the Botanical Gardens to the implementation of the Global Biodiversity Conservation Strategy targets a series of priority areas, one of which is the diversification of the gene pool of living plant collections, as well as the development of urban and rural green spaces. Green spaces are essential factors in human life and the development of public or private territories, which contribute to ensuring the conditions necessary to increase the quality of life. Moreover, the way of arranging and associating plants and the share of green spaces in urban planning constitute an indicator of the degree of civilization of a nation. Among the multitude of woody species recommended for this purpose, taxa of the genus *Cornus* L. are also listed. In recent years (2019-present), 5 species and 28 new taxa of dogwood have been mobilized and acclimatized in the NBGI (Roșca et al., 2024).

The species and taxa mobilized and conserved in the NBGI are briefly described below.

Cornus alba L. (*C. tatarica* Mill., *C. sibirica* Pall.) – the white dogwood – is a shrub that grows up to 3 m tall, occurring in Europe, Siberia and the Far East. It has erect stems, shoots with large, white pith and bright, bloody-red bark. The leaves are broadly ovate to elliptical, 7-9 cm long, rough on the ventral side, dark green, glaucous on the dorsal side with flattened hairs. The flowers of the studied taxa ('Elegantisima', 'Aurea', 'Sibirica', 'Cream Cracker' PBR), similar to the species, are white or yellowish-white, with very short sepals, ovate petals, hairy ovary (Figure 2a-2d). The flowers are united in a corymb-type inflorescence. The length of the inflorescence varies from 3.5 to 5 cm in length. The average diameter of the fruit was 5 mm. The fruit is a globose drupe, white or bluish in color depending on the taxon. It blooms in the May-June period, and the maturity of the fruits occurs in August. The taxa taken in the study similar to the species are with a high degree of decorativeness even in winter, when the leaves are missing.

Over the years in the NGBI they have been mobilized and acclimatized taxa of *Cornus alba* have adapted and acclimatized - 'Elegantissima', 'Aurea', 'Sibirica', 'Ivory Halo' PBR, 'Baton Rouge' Minbat, 'Cream Cracker' PBR, 'Siberian Pearls', *Cornus amomum* 'Blue Cloud', which have particular ornamental value due to the height and habit of the plant, the growth rate of the shoots, the striking color of the shoots against the snowy background, the foliage throughout the growing season, especially at the end of October-November. These characters amplify the aesthetic value of landscape designs. *Cornus alba* 'Elegantissima' (white dogwood 'Elegantissima') is a popular variety of dogwood, particularly prized for its elegant and attractive leaves, as well as its vibrant red stems. *Cornus alba* 'Elegantissima' has oval or elliptical leaves, bright green with white or cream edges (Figure 1a). This feature gives it a particularly attractive appearance in landscaping. The leaves remain decorative throughout the growing season. The

bright red stems are one of the distinguishing features of this dogwood variety (Figure 3a). These stems become more evident as the winter season progresses, providing a vibrant element to the winter landscape. *Cornus alba* 'Elegantissima' is a small to medium-sized shrub, reaching from 120 to 300 cm in height at maturity. In spring, this dogwood produces small, inconspicuous flowers in shades of white or cream (Figure 2a), which are followed by blue or white fruits in summer. At the end of the growing season, the leaves may turn shades of red and orange before falling, enhancing their decorativeness in late autumn.

Cornus alba 'Elegantissima' is frost hardy and can grow in temperate to cold climates. It is a versatile shrub, suitable for a wide range of climatic conditions. It prefers moist, well-drained soils, but can also tolerate clay or sandy soils. In the first years after planting, it is necessary to maintain constant soil moisture. It grows best in full sun, but tolerates partial shade. The 'Elegantissima' dogwood is relatively easy to care for. Periodic pruning of older stems can stimulate the growth of new red stems, which can improve the appearance of the landscape, maintain the shape and health of the shrub.

Plant height at the end of the fifth growing season ranged from 60 to 89 cm, with an average of 73.1 cm (Figure 4). The vibrant red stems make this shrub an excellent choice for mass planting or to create colorful hedges.

Cornus alba 'Elegantissima' is an attractive ornamental shrub that offers elegant leaves with white or cream edges, vibrant red stems, delicate flowers in spring and a variety of colors in autumn. It is low-maintenance and fits into a variety of landscape configurations. The growth of annual shoots in the fifth growing season ranged from 47 to 76 cm, with an average of 61 cm (Figure 4).



a) *Cornus alba* 'Elegantisima'



b) *Cornus alba* 'Aurea'



c) *Cornus alba* 'Sibirica'



d) *Cornus alba* 'Cream Cracker' PBR

Figure 1. Taxa of *Cornus alba* L. in the fifth growing season

Cornus alba 'Aurea' is a shrub with golden leaves, showy throughout the growing season (Figure 1b). The common name for this variety is Aurea dogwood. The stems are reddish-brown (Figure 3b). The red stems are often used in the art of making bouquets in floristry.

The leaves are ovate in shades of green to yellowish green. The Aurea dogwood blooms in May and June. The small, creamy white flowers appear in early summer (Figure 2b), followed by clusters of small white fruits. It is a medium-sized shrub that reaches 200-300 cm in height at

maturity. It grows best on well-drained, sandy, loamy, acidic soils. Its shiny golden leaves and red branches in winter bring a touch of color and elegance to any space. It is low-maintenance and adapts to different environmental conditions.
It is planted alone or as a hedge, in groups and in combination with other species to create spectacular color contrasts throughout the growing season.
The height of the shrub in the fifth growing season varied between 97 and 165 cm, with an average of 119.8 cm. The growth of the shoots by the end of the growing season varied between 46.5 and 127 cm, with an average of 97.95 cm (Figure 4).



a) *Cornus alba* 'Elegantisima'



b) *Cornus alba* 'Aurea'



c) *Cornus alba* 'Sibirica'



d) *Cornus alba* 'Cream Cracker' PBR

Figure 2. Taxa of *Cornus alba* L in the flowering phase

Cornus alba 'Sibirica' (Siberian dogwood) is a medium-sized shrub. In the fifth growing season, the plant height reached values between 87 and 161 cm, the average being 127.6 cm after annual shaping cuts (Figure 4). Mature shrubs reach 150-200 cm in height. The most distinctive feature of the studied taxon is its multiple bright red stems, of particular decorative value in winter (Figure 3c). The growth rate is fast, ranging from 67 to 133 cm, with an average of 94.3 cm, (Figure 4) depending on the compliance with the recommended technology, genotype and climatic conditions during the growing season. The leaves are medium-sized, oval or elliptical, dark green in summer (Figure 1c), may turn red or purple in autumn, adding another level of decorativeness and seasonal interest. During spring, the plant produces small, white flowers with a yellowish tinge, but the blooms are not as spectacular as compared with other dogwood varieties (Figure 2c). The fruits are small, bluish. It is frost-hardy, immune to diseases and pests, adapts to various soil types, but prefers fertile soil, tolerates both full sun and partial shade. It is relatively easy to maintain, only shape-correcting pruning may be needed. Periodic pruning, like in all the studied taxa, was carried out in spring to maintain decorativeness during winter, created through the vibrant color of the stems which contrasts perfectly with the immaculate white snow, and to stimulate the growth of annual vines.



Cornus alba 'Elegantisima' *Cornus alba* 'Aurea' *Cornus alba* 'Sibirica' *Cornus alba* 'Cream Cracker' PBR

Figure 3. The general aspect of annual shoots in the winter season in *Cornus alba* L. taxa

Cornus alba 'Cream Cracker' PBR similar to other taxa, it is medium-sized with a high degree of decorativeness through various characters throughout the growing season. The round crown, with purple-red annual and biennial shoots, maintains its decorative effect during winter (Figure 3d).

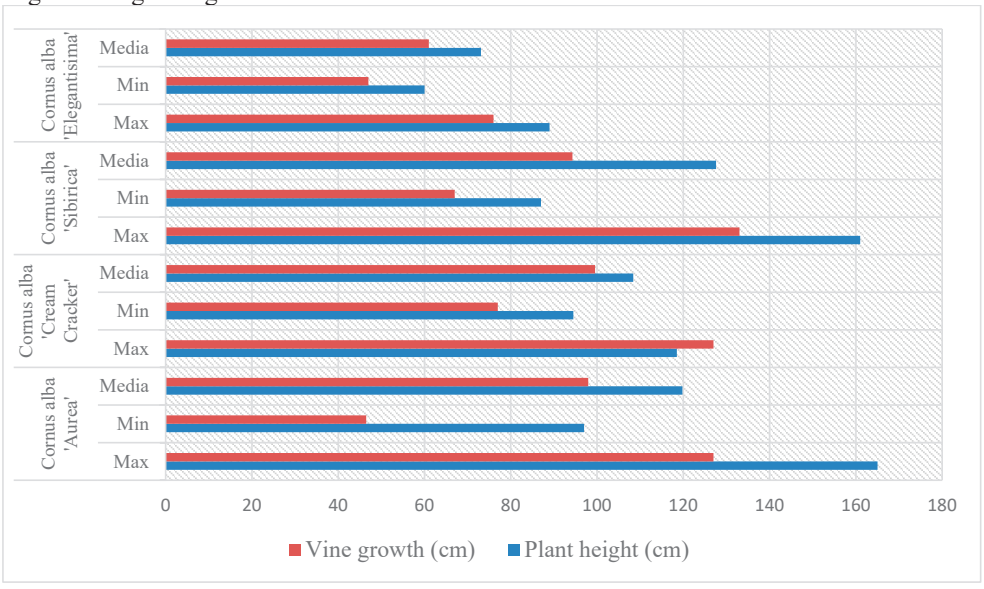


Figure 4. Morphological parameters in the fifth growing season of *Cornus alba* L. taxa

In the fifth growing season, the plant height varied between 94.5 and 118.5 cm, with an average of 108.4 cm (Figure 4). At maturity it reaches 200-250 cm. This taxon is spectacular in early spring with its young leaves with white edges, which turn yellow at maturity (Figure 1d). The rapid growth rate ranged from 77 to 127 cm, the average was 99.6 cm (Figure 4). It blooms profusely in June, and sometimes it blooms for the second time in August-September. The small, creamy flowers are grouped in corymb inflorescences (Figure 2d).

The fruits are small, white and few in number, depending on the climatic conditions at that time. It is resistant to frost, pollutant substances, diseases and pests. It prefers moist, drained, loose, slightly acidic soils and is unpretentious to soil fertility. It tolerates shade, but prefers sunny places. Similar to other taxa, it is planted at a distance of 60-100 cm in autumn or spring. The survival rate of transplanted seedlings is higher when they are with packed roots, protected or in a container.

CONCLUSIONS

The collection of the genus *Cornus* L. of the NBGI was diversified with 5 species and 28 new ornamental non-native taxa and totals 8 species and 28 taxa. The introduced species taken into the studio (*Cornus alba* 'Elegantisima', *Cornus alba* 'Aurea', *Cornus alba* 'Sibirica', *Cornus alba* 'Cream Craker' PBR) are varieties with high ornamental potential through various characteristics and are distinguished by diverse habit, intense colors of shoots, leaves, fruits, period and abundance of flowering, fruiting, as well as the ability to adapt to soil and climatic conditions.

The studied cultivars are resistant to drought, frost and polluting agents, and do not require special care, only sanitary and shaping pruning, as well as compliance with the recommended technology throughout the growing season.

The varieties researched of *Cornus* L. (*Cornus alba* 'Elegantisima', *Cornus alba* 'Aurea', *Cornus alba* 'Sibirica', *Cornus alba* 'Cream Craker' PBR) They are decorative due to the shape and size of their habit, the color of their shoots particularly showy during the winter, the shade of their foliage throughout the growing season, especially in late autumn, and the abundance of their flowering.

It is recommended for use in the landscape design of green spaces, in the foreground in small groups, in border mixes and for hedges of various heights and colors.

The study of these taxa contributes to the diversification of the dendrological gene pool of the Republic and to the implementation of biodiversity conservation strategies, demonstrating that the varieties of *Cornus alba* L. are promising and are recommended for large-scale use in rehabilitation and landscaping projects of green spaces in the 3 dendrological districts of the Republic of Moldova.

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BIOLOGICAL, ECOLOGICAL AND MORPHOLOGICAL FEATURES OF SAKURA (*PRUNUS SERRULATA* L.*)* CULTIVATION FOR FURTHER USE IN LANDSCAPING OF UKRAINE

Yuliya STRUTYNSKA[1], Olena OLESHKO[1], Tetiana LOZINSKA[1], Vladyslav MASALSKYI[1], Mykola GRABOVSKYI[1], Valentyn POLISHCHUK[2], Anatolii BALABAK[2], Iryna KOZACHENKO[2]

[1]Bila Tserkva National Agrarian University, Soborna Square 8/1, Bila Tserkva, Kyiv region, 09110, Ukraine
[2]Uman National University of Horticulture, Instutytsjka street 1, Uman, Cherkasy region, 20305, Ukraine

Corresponding author email: nikgr1977@gmail.com

Abstract

At present, a great deal of experience has been gained in landscaping and gardening of cities and villages, a rich assortment of landscaping plants has been created and agricultural techniques for their cultivation have been developed, the necessary methods of landscaping specific to cities have been found, and methods of maintaining green spaces have been determined. However, amateur gardeners and professionals working in the field of landscape art are interested in introduced woody ornamental plants that are characterized by high decorative value and have a specific growing season. One of these plants is sakura, a representative of the Prunus L. family - Prunus serrulata L. The main arguments for this are the high decorative qualities of sakura, their resistance to urban conditions, and rapid adaptation to new cultivation conditions. The article presents a brief morphological classification of the most common genotypes of the genus Prunus serullata L. in Ukraine. The biological features of growth, seasonal rhythms of sakura plants development are investigated.

Key words*: sakura, varieties, flowering, cultivation, landscaping.*

INTRODUCTION

The Chinese flowering cherry, also known as sakura or cherry blossom, is an ornamental tree species that blooms beautifully in spring and is globally popular (Shirasawa et al., 2022). The term "sakura" is commonly used to describe deciduous trees belonging to the Rosaceae family (Shibato et al., 2019). Since sakura is a variety of plant species, it has flowers of different colors from pure white to bright pink. But most often, sakura is understood as a representative of the cherry subgenus, especially the fine-pollinated cherry. In 1963, the book "Garden Plants of Japan" was published, compiled by dendrologists from the University of Tokyo.

According to this publication, sakura includes the following species: mountain sakura (*P. Jamasakura*), Edo cherry (*P. yedoensis*), short-bristled cherry (*P. subhirtella*), Sargent cherry (*P. sargentii*), glandular cherry (*P. glandulosa*), bell cherry (*P. campanulata*), fine-sawed cherry (*P. serrulata*), and sharp-sawed cherry (*C. serrulata*) (McClellan, 2005; Moriuchi & Basil, 2019).

The genus *Prunus* L. now unites about 230 species that once belonged to the independent genera *Amygdalus* L. (almond), *Armeniaca* Scop. (apricot), *Cerasus* Mill. (exochorda), *Laurocerasus* Duhamel (laurel), *Maddenia* Hook.f. & Thomson (maddenia), *Oemleria* Rchb. (emberia), *Padus* Mill. (bird cherry), *Persica* Mill. (peach), *Prinsepia* Royle (prinsepia), *Pygeum* Gaertn. (African plum) and some others (Aranzana et al, 2019).

Attempts to taxonomically organize the composition of *Prunus* L. have been going on for more than 300 years, but there is still no consensus on the system of this rather polymorphic genus. It belongs to the Rosaceae family (Perez Zabala, 2022). This is a large family of grasses consisting of three subfamilies: dewy, amygdaloid, and dry, with about 3000 species. The Rosaceae plant family includes grasses, shrubs, and trees, and has a large number of hybrids of different ploidy. The classification of plants belonging to the genus

Prunus L. is quite controversial. These are genotypes of not only cherries, but also plums, cherries, and even bird cherry (Chin et al., 2014).

Prunus serrulata with several intraspecific taxa, including *P. jamasakura* and *P. leveilleana*, is widespread in East Asia. Despite the fact that these taxa are very common, the taxonomic confusion surrounding this complex is reflected in the ambiguity demonstrated by the different taxonomic treatments currently in use. Patterns of intraspecific variability and taxon recognition in the *P. serrulata* complex and related species were investigated on 468 individuals using principal component analysis. Particular emphasis was placed on *P. serrulata*, *P. jamasakura*, and *P. leveilleana* due to their high degree of morphological integration and inconsistent restriction in different systematic treatments. Although morphological variation among different taxa of the *P. serrulata* complex was constant for most reproductive and leaf characters, related taxa (*P. pseudocerasus*, *P. lannesiana* f. *albida*, and *P. sargentii*) appeared to differ in terms of the number of flowers per inflorescence and peduncle length. In general, the number of flowers per inflorescence, flower size, peduncle length, and pedicel hair density are characters of high taxonomic value for most related taxa. However, there is insufficient morphological evidence for the taxonomic division of *P. serrulata* to justify the definition of varieties, with the exception of the pubescent taxon *P. serrulata* var. *pubesecens* (Chang et al., 2007).

Prunus serrulata is native to Japan, Korea and China. The plant is widespread in the western and eastern regions of China, and has also been recorded on the Korean peninsula and in Japan (Iwatsuki et al., 1993; Yi et al., 2020a). The Japanese have established two terms to distinguish wild cherries from cultivated or orchard cherries. These are "Yama-zakura" (mountain cherry) for wild plants and "Sato-zakura" (village cherry) for cultivars. The true non-breeding species *P. serrulata* is rarely sold commercially because it does not have the bright floral traits that highly bred varieties have. It is believed that actively cultivated village cherries began to be grown around the beginning of the seventeenth century (Wybe, 1999), although some varieties may date as far back as 794 and 1192 AD (Honda & Hayashi, 1974). Today, more than 600 varieties of *P. serrulata* are known in Japan, producing bright flowers in spring and beautiful leaf color in autumn. Their exact origin remains rather uncertain. Most of the semi-double forms have certain consistent botanical characteristics that point to the Oshima cherry (*P. speciosa*) as the parent. This species is believed to have a tendency to mutate into double-flowered varieties (Hurr et al., 2022). Many regions have introduced cherry varieties and researched plant materials from the genus *Prunus* to counteract the monotony that comes after a landscape with cherry blossoms loses its appeal. For centuries, the Japanese have been working on creating new forms of sakura. They have achieved amazing results, as there are plants with double flowers (up to 50 petals) that resemble peonies or chrysanthemums and reach about 6 cm in diameter (Liu et al., 2020).

Despite their uncertain origins, most semi-double forms share some consistent botanical characteristics. Therefore, these forms were grouped as varieties in 1916 by the Japanese botanist Manabu Miyoshi and his American colleague Ernest H. Wilson (Akai, 1974). The Flower Association of Japan, for which the word cherry blossom means the first "cherry blossom," prepared a detailed descriptive manual in 1982 of nearly two hundred species, varieties, and hybrids of Japanese flowering cherries. One of the researchers in the association was the scientist T. Kawasaki, who published Nihonno Sakura (Flowering Cherries of Japan) in 1993. This shows an even more thorough understanding of cherry blossoms and attempts to roughly classify almost 350 cherries into several groups, similar to family trees (Sakurai, 2017).

The Chinese flowering cherry tree has significant economic value due to its great appeal to tourists during the spring bloom. In addition to its aesthetic appeal, it also offers a range of medicinal benefits, including anti-inflammatory properties for the skin, prevention of skin roughness, and inhibition of melanin production. The plant is also used as an ingredient in tea (Shimoda et al., 2011). The polyphenols contained in cherries also help to increase abnormal serum lipid levels, regulate blood glucose levels, and reduce obesity (Yi et al., 2020b). Its beautiful flowers, wide adaptability and high decorative value contri-

bute to its widespread use in urban and landscape design (Wang, 2014).

The *Prunus serrulata* species is well adapted to various climatic conditions (Zhi-xiong et al., 2010). Due to the wide variation in its traits and flower color variations, *P. serrulata* serves as the parent of many ornamental cherry species and is a very important germplasm resource for floral species (Yi et al., 2020a). Using *P. serrulata* as a parent plant, a variety of natural and artificial hybrids have been developed and selected (Ma et al., 2009). Without pruning and under favorable conditions, this cherry tree grows to the size of a full-fledged tree, more than 20 meters high. The Japanese sakura is grown for its beautiful flowers, which appear on the branches every spring and cover almost the entire plant with a pink double blanket. Due to its luxurious flowering, the sakura tree has become widespread in many countries (Salazar, 2022; Kuitert et al., 1999). Some of the cultivated species of *Prunus serrulata* exist as a single monumental specimen or a small group of trees in specialized collections, and are unlikely to deserve cultivar status. Others are well described and commonly found in many parts of the world.

The use of flowering cherries to enhance suburban landscapes and attract public recreation has significant economic and social value. However, the current quality of the cherry landscape is compromised due to a lack of variety selection and planting and design planning. Based on the analysis of public preferences, there is great significance to improve the sustainability of the cherry blossom landscape. The results of the scenic beauty model showed that the cleanliness of the scene (38.04%), the color composition of the plants (25.59%), the road (17.79%), the type of plant (12.88%) and the building (2.70%) were the five key factors affecting the scenic beauty. These study results provide objective reference information for future research on cherry blossom, as well as landscape planning, design, and management (Peng et al., 2024).

Sakura plants should be planted in spring or June, as the seedlings need a long time to adapt. To increase the survival rate of planting material, it should be planted on the south side. Sakura trees prefer an elevated location and grow well on gentle (no more than 10°C) slopes. Damp lowlands, hollows, and forest glades with poor air aeration are not suitable for planting a garden. The root system is not very deep, so to prevent flooding, it is recommended to plant sakura in low-lying areas (Opalko et al., 2016). Sakura trees grow very slowly, so it is advisable to form their crown in 8-10 years. Sanitary pruning is carried out after 2-3 years, removing dry, broken branches and those that show signs of pests and diseases in the spring before the sap flow begins (Polishchuk & Shcherba, 2016). In order to protect plants from diseases, it is advisable to collect and dispose of fallen leaves in the fall. Watering should be done only on the driest days. It is recommended to loosen the soil around the trunks of young plantings, remove weeds and mulch the soil under the plant to protect the roots from overheating and drying out. Sakura looks great both in a single planting: on a lawn in a prominent place, and in various landscape compositions (Frazier, 2014; Ohnuki-Tierney et al., 1998).

The 'Kanzan' variety is very popular in landscape architecture (Maurer U et al., 2003). The 'Shirotae' and 'Taihaku' varieties should be used along pedestrian paths and in places such as shopping centers and hospitals (Forrest, 2006). Sakura is resistant to mild air and soil pollution, but is susceptible to brown stone fruit rot (*Monilia laxa* (*Ehrenb.*) *Sacc.*) if not properly maintained (Ninić-Todorović et al., 2012).

Ukraine has adopted the Japanese tradition of planting sakura on city streets. In recent years, there has been a significant increase in demand for ornamental crops, including sakura planting material. Sakura plants successfully overwinter in Kyiv, Lviv, Zakarpattia, and Moldova. There have been attempts to grow small-saw cherries in Estonia and Latvia, but the climate of northern latitudes is not very suitable for them (Opalko et al., 2018). Today, there are many winter-hardy varieties of sakura, which makes it possible to cultivate it in Ukraine (Polishchuk & Shcherba, 2016; Komar-Temna, 2013). At the same time, the development of varieties with early and stable multi-season flowering and the development of appropriate agricultural practices are two ways to solve the problem of short-term and concentrated flowering of sakura (Xu et al., 2023).

The analysis of literature sources shows that varieties of the genus *Prunus* L. are used for landscaping in Ukraine, but there is almost no

scientific literature on the regularities in the rhythm of development, methods of cultivation and possibilities of using sakura varieties for landscaping and gardening, taking into account the peculiarities of the flowering phase.

MATERIALS AND METHODS

The research on the biological, ecological and morphological features of sakura (*Prunus serrulata* L.) cultivation was conducted in 2021-2023 in the botanical garden of the Uman National University of Horticulture. They were carried out according to the Methodology for the examination of plant varieties of the ornamental group for distinctiveness, uniformity and stability, edited by Tkachuk (2016). According to this methodology, the following main phases of sakura vegetation are distinguished the beginning of shoot growth, bud swelling - an increase in their size, the beginning of flowering, the beginning of leafing - the first leaves have unfolded and are similar in shape to the leaves of this species, complete leafing - all leaves acquire normal size, beginning of leaf yellowing - appearance of the first leaves with a changed autumn color, complete leaf yellowing - yellowing of the main part of the leaf mass, beginning of leaf fall - fall of single leaves, complete completion of autumn leaf fall. The flowering phase begins with the blooming of flowers and when about 25% of the buds have blossomed on the tree, the beginning of the flowering phase is recorded, after 75% mass flowering and during the period of 25% of the flowers petals have fallen off - the end of flowering. Determining the phenological phases of development of plants of the genus *Prunus* L., and especially the flowering phases, makes it possible to form different options for landscaping the territory.

Among the varietal diversity of the genus *Prunus* L., special attention should be paid to the varieties *P. serrulata* '*Amanogawa*', *P. serrulata* '*Kiku Shidare*', *P. serrulata* '*Kanzan*' and *P. serrulata* '*Royal Burgundy*', which have successfully passed adaptation and are introduced woody plants in Ukraine. The adaptation of plants to environmental conditions depends largely on their biological characteristics and ability to grow and develop in new soil and climatic conditions. Changes in the growing conditions of ecotypes, compared to the optimal ones, lead to deviations in plant development from the typical ones that are characteristic of the natural range. These changes are especially noticeable between the vegetative development of plants and the formation of generative organs.

The research was conducted with plants of the genus *Prunus serrulata* L., which were bred in Japan, of the following varieties.

The 'Amanogawa' variety

A small tree with a dense columnar crown (Figure 1). Reaches a height of 5 m and a width of 1-2 m. It blooms in May with light pink double flowers 4-5 cm in diameter, collected in bunches. Due to its rather compact size, it can be planted in small gardens as an accent in the rabatka.



Figure 1. Sakura plant of the 'Amanogawa' variety

The 'Kanzan' variety

The tree is medium-sized with a reverse pyramidal crown (Figure 2). Each inflorescence consists of 3-5 large densely double deep pink flowers with a purple tint. It blooms from mid-April to the second decade of May. Can be used for tapeworms, group and alley plantings.



Figure 2. Sakura plant of the 'Kanzan' variety

The 'Kiku Shidare' variety weeping form

A small tree with weeping branches and an openwork crown (Figure 3). Reaches a height of 4 m and a width of 3 m. The flowers are double, pink, 6 cm in diameter, collected in bunches of several pieces. Flowering is very abundant. Prefers sun, fertile and moist soils. It is advisable to plant in places protected from cold winds.



Figure 3. Sakura plant of the 'Kiku Shidare' variety

The 'Royal Burgundy' variety

A medium-sized tree with an inversely conical crown and red leaves (Figure 4). The flowers are double, purple-pink, up to 6 cm in diameter, hanging on long pedicels, collected in bunches. It looks spectacular in compositions, alleys, as an accent plant on the lawn.



Figure 4. Sakura plant of the 'Royal Burgundy' variety

All the varieties were brought from the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine and the Sofiyivka National Dendrological Park of the National Academy of Sciences of Ukraine and planted on the territory of the Uman National University of Horticulture for further research.

RESULTS AND DISCUSSIONS

The flowering of cherry trees is an effective indicator of climate change impacts on phenology, as the timing of their flowering is highly sensitive to temperature, especially in winter and early spring (i.e. February and March) (Miller-Rushing et al., 2007). From a cultural point of view, accurate prediction of cherry phenology is crucial, as many spring festivals and events around the world are timed to coincide with a specific phenological event - the peak bloom date (PBD).

The blossoming of flowering cherry species (e.g., *Prunus serrulata*, *Prunus* × *yedoensis*, and *Prunus subhirtella*) is celebrated with festivities in many parts of the world, including the United States, Europe, and Asia (e.g., Washington, D.C., USA; Tokyo and Kyoto, Japan; Jinhae and Seoul, Korea). These spring cherry blossom festivals are culturally and economically important events, and successful planning requires that the cherry blossoms appear as expected during the festival period. In a rapidly changing climate, predicting bloom dates based solely on past history is likely to become less reliable; therefore, a more robust predictive model is needed not only for planning purposes of these cultural events, but also for assessing the agricultural and environmental impacts of climate change (Chung et al., 2011).

Flowering of woody plants is influenced by various factors, including temperature, hormones and nutrition, each affecting plant growth and development to a different degree. Temperature plays an important role in inhibiting plant growth and development. Exposure to low temperatures can damage plant structures, induce stress responses, activate the expression of relevant genes, change the composition and structure of cell membranes, and lead to the accumulation of osmotic regulatory substances to counteract the physical and physiological damage caused by low temperatures (Vosnjak et al., 2021; Kose et al., 2022; Shi et al., 2017). When plants reach the stage of flower bud development, carbohydrates become the fundamental building blocks for converting nutritive growth into reproductive growth. The accumulation of carbohydrates promotes flower bud differentiation (Kataoka et al., 2004). Soluble sugars and soluble proteins in

their leaves are continuously transported to flower buds, providing the necessary substances and energy for bud differentiation (He et al., 2018). The distribution pattern of carbohydrates, proteins, and other nutrients is also closely related to flowering (Zheng et al., 2019).

According to the results of phenological observations, it was found that the vegetation period of *Prunus* L. varieties 209-214 days over the years of research (Figure 5).

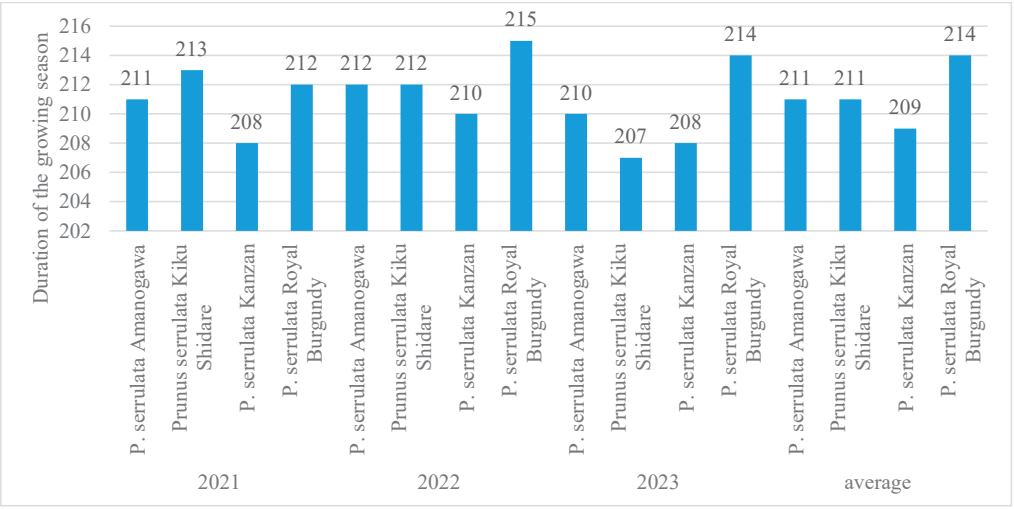


Figure 5. Duration of the growing season of *Prunus* L. varieties (2021-2023), days

No significant change in the length of the growing season was observed by the years of vegetation: if in 2021 the duration of the growing season of *P. serrulata 'Amanogawa'* was 211 days, in 2022 - 212 days, and in 2023 - 210 days. Similar results were obtained for other varieties.

There were no significant deviations in the length of the growing season by variety. On average, over the years of research, the shortest vegetation period of 209 days was in the variety *P. serrulata 'Kanzan'*, the longest - 214 days in the variety *P. serrulata 'Royal Burgundy'*.

In the conditions of the Right-Bank Forest-Steppe of Ukraine, the vegetation of plants of the genus *Prunus* L. began at an average monthly air temperature of +7.4°C (2021), +8.6°C (2022), +8.3°C (2023), in conditions close to the average perennial.

The beginning of the growing season in spring is manifested by the beginning of shoot growth and the extension of bud scales, which protected the plant from negative influences during the autumn, winter and early spring dormant periods.

The phase of bud swelling - increase in their size of all species of *Prunus serrulata 'Amanogawa'*, *Prunus serrulata 'Kiku Shidare'*, *Prunus serrulata 'Kanzan'*, *Prunus serrulata 'Royal Burgundy'* took place in the late second - early third decades of April. No significant difference in the passage of the bud swelling phase was found both by varieties and years of study. The earliest bud swelling was noted in the *Prunus serrulata 'Royal Burgundy'* variety: in 2021 - April 18, in 2022 and 2023 - April 16, the latest in the *Prunus serrulata 'Amanogawa'* variety, respectively - April 20, 22 and 19 (Tables 1-3).

Table 1. Passage of phenological phases of development of plants of the genus *Prunus serrulata* L., 2021

Phenological phases	Date of observation by variety			
	P. serrulata 'Amanogawa'	*P. serrulata 'Kiku Shidare'*	*P. serrulata 'Kanzan'*	*P. serrulata 'Royal Burgundy'*
1	2	3	4	5
The beginning of the growing season	02.04.2021	03.04.2021	05.04.2021	02.04.2021
The beginning of shoot growth	10.04.2021	11.04.2021	15.04.2021	11.04.2021

Kidney swelling - an increase in their size	20.04.2021	19.04.2021	19.04.2021	18.04.2021
The beginning of flowering	05.05.2021;	07.05.2021;	10.05.2021;	06.05.2021
Beginning of balding - the first leaves have unfolded and are similar in shape to the leaves of this species	08.05.2021	11.05.2021	15.05.2021	14.05.2021
Complete balding - all leaves acquire normal size	22.05.2021	22.05.2021	23.05.2021	23.05.2021
Beginning of leaf yellowing - appearance of the first leaves with a changed autumn color	18.09.2021	20.09.2021	20.09.2021	17.09.2021
Complete yellowing of leaves - yellowing of the main part of the leaf mass	30.09.2021	29.09.2021	30.09.2021	01.10.2021
Beginning of leaf fall - fall of single leaves	15.10.2021	17.10.2021	17.10.2021	19.10.2021
Complete completion of the autumn leaf fall	30.10.2021	02.11.2021	30.10.2021	02.11.2021
Duration of the growing season	211	213	208	212

Table 2. Passage of phenological phases of development of plants of the genus *Prunus serrulata* L., 2022

Phenological phases	Date of observation by variety			
	P. serrulata 'Amanogawa'	*P. serrulata 'Kiku Shidare'*	*P. serrulata 'Kanzan'*	*P. serrulata 'Royal Burgundy'*
The beginning of the growing season	04.04.2022	06.04.2022	05.04.2022	01.04.2022
The beginning of shoot growth	12.04.2022	14.04.2022	13.04.2022	10.04.2022
Kidney swelling - an increase in their size	22.04.2022	21.04.2022	20.04.2022	16.04.2022
The beginning of flowering	06.05.2022;	08.05.2022;	09.05.2022;	05.05.2022;
Beginning of balding - the first leaves have unfolded and are similar in shape to the leaves of this species	10.05.2022	12.05.2022	14.05.2022	12.05.2022
Complete balding - all leaves acquire normal size	25.05.2022	25.05.2022	23.05.2022	22.05.2022
Beginning of leaf yellowing - appearance of the first leaves with a changed autumn color	20.09.2022	23.09.2022	23.09.2022	18.09.2022
Complete yellowing of leaves - yellowing of the main part of the leaf mass	02.10.2022	30.09.2022	01.10.2022	02.10.2022
Beginning of leaf fall - fall of single leaves	16.10.2022	20.10.2022	18.10.2022	16.10.2022
Complete completion of the autumn leaf fall	02.11.2022	03.11.2022	01.11.2022	01.11.2022
Duration of the growing season	212	212	210	215

Observations of the phenological phases of sakura plant development showed that the beginning of the plant vegetation and flowering depended on the sum of effective temperatures, which was 106.5-299.0°C and occurred in the first decade of April and the first decade of May, respectively.

Table 3. Passage of phenological phases of development of plants of the genus *Prunus serrulata* L., 2023

Phenological phases	Date of observation by variety			
	P. serrulata 'Amanogawa'	*P. serrulata 'Kiku Shidare'*	*P. serrulata 'Kanzan'*	*P. serrulata 'Royal Burgundy'*
The beginning of the growing season	07.04.2023	04.04.2023	05.04.2023	01.04.2023
The beginning of shoot growth	14.04.2023	12.04.2023	13.04.2023	10.04.2023
Kidney swelling - an increase in their size	19.04.2023	18.04.2023	20.04.2023	16.04.2023
The beginning of flowering	05.05.2023	04.05.2023	09.05.2023	05.05.2023
Beginning of balding - the first leaves have unfolded and are similar in shape to the leaves of this species	10.05.2023	09.05.2023	14.05.2023	12.05.2023
Complete balding - all leaves acquire normal size	23.05.2023	22.05.2023	23.05.2023	22.05.2023

Beginning of leaf yellowing - appearance of the first leaves with a changed autumn color	18.09.2023	21.09.2023	23.09.2023	18.09.2023
Complete yellowing of leaves - yellowing of the main part of the leaf mass	02.10.2023	29.09.2023	01.10.2023	02.10.2023
Beginning of leaf fall - fall of single leaves	14.10.2023	18.10.2023	18.10.2023	16.10.2023
Complete completion of the autumn leaf fall	01.11.2023	02.11.2023	01.11.2023	01.11.2023
Duration of the growing season	210	208	208	214

The researchers established models of linear and nonlinear relationships between flowering periods and climatic factors by analyzing trends in flowering periods and their correlation with climatic factors. They summarized the relationship between cherry blossom periods and winter temperature changes, contributing to the prediction of flowering periods and the proper planning of cherry blossom landscapes (Chen et al., 2008; Shu et al., 2018; Tan et al., 2021). Previous studies have analyzed the cherry blossom periods and morphological characteristics of cherry blossom landscapes to create an optimal cherry blossom viewing experience. The analysis was based on combinations of flowering periods and general patterns based on different flowering periods and morphological characteristics of different cherry varieties (Chen, 2022). Under Korean conditions, Japanese cherry blossom is expected to bloom 9, 21, and 29 days earlier in the future normal years 2011-2040, 2041-2070, and 2071-2100, respectively. In the southern coastal areas, incomplete or no flowering of Japanese cherries may be observed in spring due to insufficient cooling to interrupt bud dormancy (Jin-I, 2006). On average, over the three years of research, the duration of flowering within each variety did not change significantly. The longest flowering period was observed in the varieties *P. serrulata 'Kiku Shidare'* (21 days) and *P. serrulata 'Kanzan'* (20 days), the shortest in the varieties *P. serrulata 'Amanogawa'* (18 days) and *P. serrulata 'Royal Burgundy'* (18 days). The duration of flowering depended on the weather conditions of the growing season: in 2021, on average, it was 19 days for varieties, in 2022 - 20 days, in 2023 - 18 days (Figure 6).

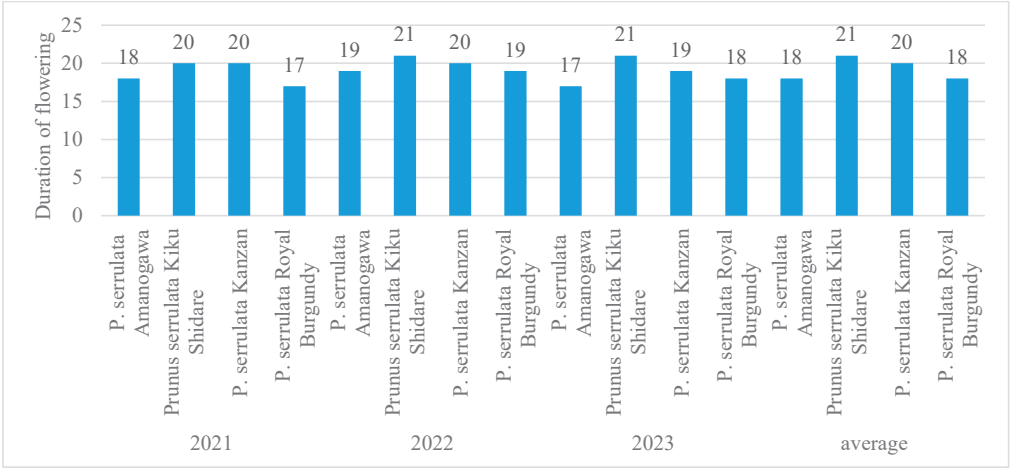


Figure 6. Duration of flowering depending on varietal characteristics and growing conditions, days

Slight differences over the years were found in the duration of the flowering phase of plants, which is associated with varietal characteristics and average daily air temperatures. Thus, in 2021, 2022 and 2023, the longest flowering duration of 20-21 days was in the variety *P. serrulata 'Kiku Shidare'*, and the shortest duration of 17-19 days in the variety *P. serrulata 'Royal Burgundy'*. Taking into account the beginning of flowering and its duration of these varieties, it is possible to form landscape compositions, namely: planting sakura varieties *P. serrulata 'Amanogawa'* and *P. serrulata 'Royal Burgundy'*, in which the flowering phase begins the earliest, but its duration is the shortest, along with the varieties *P. serrulata*

'Kiku Shidare' and *P. serrulata 'Kanzan'*, whose flowering begins later and its duration is longer, which will ensure the continuation of flowering in the composition.

Previously, several studies (Cesaraccio et al., 2004; Jung et al., 2005) showed that a two-stage thermal time-based phenological model successfully predicted the flowering time of temperate tree species, including fruit crops and flowering cherries such as *Prunus serrulata var. spontanea*. Chung et al. (2009) extended the model for native flowering cherries in Korea and applied it to predict current and future flowering dates in South Korea. Under the A2 emissions scenario, the peak flowering of South Korean cherry trees is likely to occur much earlier by an average of 21 days by 2050. At the regional scale, the spatial variability of projected flowering dates increased in the late 21st century, but overall it is projected that the peak flowering is likely to occur 29 days earlier on average by 2080 (Chung U et al., 2009).

The peak bloom dates (PBD) of flowering cherry trees (*Prunus×yedoensis 'Yoshino'* and *Prunus serrulata ''Kanzan"*) in Washington, DC are likely to accelerate by an average of five days by 2050 and 10 days by 2080 for these cultivars under the mid-range (A1B) emissions scenario projected by the ECHAM5 general circulation model. The acceleration is likely to be much greater (13 days for 2050 and 29 days for 2080) under the higher (A2) emissions scenario projected by the CGCM2 general circulation model. These results demonstrate the potential impacts of climate change on cherry blossom timing and illustrate the usefulness of a simple process-based phenological model for developing climate change adaptation strategies in horticulture (Chung et al., 2011).

According to our observations, the beginning of leaf fall - the first leaves that are similar in shape to the leaves of this species, depending on the varietal characteristics begins 3-8 days after the beginning of flowering and on average, in 11-12 days complete leaf fall occurs in varieties.

The beginning of the end of the sakura plant vegetation is the yellowing of the leaves, which began in the beginning of the third decade of September and only varieties in which the flowering phase begins earlier and its duration is shorter*, P. serrulata 'Amanogawa'* and *P. serrulata 'Royal Burgundy'* - at the end of the second decade of September. Full yellowing of the leaves occurs almost simultaneously in all varieties studied - in the first days of October. Over the years of research, no special differences in the passage of these phases of development have been found. In the second decade of October, leaf fall begins, which ends in early November. Depending on the varietal characteristics, there are significant differences both with the beginning of the leaf fall and with complete leaf fall.

CONCLUSIONS

Studies have shown that the introduced plants of the genus *Prunus serrulata* L. are promising for creating plant compositions in the conditions of the Right-Bank Forest-Steppe of Ukraine. The main arguments for this are the high decorative qualities of sakura, their resistance to urban conditions, and rapid adaptation to new cultivation conditions.

It was found that the growing season of *Prunus* L. varieties averaged 209-214 days in 2021-2023. Observations of the passage of phenological phases of sakura plants development made it possible to establish patterns in the rhythm of their development, their dependence on both varietal characteristics and weather conditions of the growing season. It was found that the beginning of the plant vegetation and flowering depended on the sum of effective temperatures, which was 106.5-299.0 °C and occurred, respectively, in the first decade of April and the first decade of May.

We recommend to take into account the beginning of flowering of varieties and the period of its passage, which will ensure the extension of flowering in the garden for 8-10 days and planting sakura varieties *P. serrulata 'Amanogawa'* and *P. serrulata 'Royal Burgundy'* in which the flowering phase begins earlier, and in varieties *P. serrulata 'Kiku Shidare'* and *P. serrulata 'Kanzan'* flowering begins later. Accordingly, the duration of flowering of the first two varieties is shorter compared to the last two.

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EFFICACY OF BIO-INSECTICIDES AGAINST THRIPS (THYSANOPTERA: THRIPIDAE) AS A PEST OF *PELARGONIUM* CROPS IN A COMMERCIAL GREENHOUSE IN WESTERN ROMANIA

Ana-Maria VÎRTEIU, Marius BOLDEA, Ramona ȘTEF, Florin PRUNAR, Silvia PRUNAR, Ioana GROZEA

University of Life Science "King Mihai I" from Timisoara, 119 Calea Aradului, Timişoara, Romania

Corresponding author emails: anamariavarteiu@usvt.ro, ioanagrozea@usvt.ro

Abstract

The economic importance of thrips in the last ten years, in terms of both direct (often quality) and indirect (via viruses and phytoplasmas) damage to protected flower crops, in particular pelargoniums, has in most cases necessitated the adaptation of integrated management practices. A rich thrip fauna, with Frankliniella occidentalis, Frankliniella intonsa, Thrips tabaci, Thrips major and Scirtothrips dorsalis being the most abundant species, was revealed by the analysis of the data collected from the greenhouse in Dudeștii Noi (Timiș County). Feeding pattern analysis revealed the consistent presence of very high levels of polyphagous species. In order to maintain a high quality of Pelargomium plants for rapid commercialisation, treatments were carried out in 2024 and the efficacy of bio-insecticides to control thrips species was evaluated. The results showed that the chemical products had the highest efficacy, namely Exalt with the active ingredient spinetoram and among the biological products used, Laser 240 SC (spinosad as active ingredient) had the highest efficacy. The least effective products used to control thrips were those based on diatom powder and neem oil.

Key words*: bio - insecticides, thrips, pelargonium, greenhouses, western Romania.*

INTRODUCTION

With about 280 species, the genus *Pelargonium* is the second largest genus in the family *Geraniaceae*. Most of the species are widely used as ornamental and medicinal plants (Van der Walt, 1984; Blerot et al., 2016; Celi et al., 2024).

There are many reasons why this plant is so popular, but here are just some: it is extremely easy to grow, which makes it ideal for the novice gardener; it flowers for a very long time, from spring to autumn; it comes in a rainbow of hues, from pink and red to white, orange, purple and a host of bicoloured, patterned and unusual varieties; it is available in scented, upright, climbing and many other varieties; it is versatile and can be combined with many other plants (Yu et al., 2016; Negro et al., 2021; Karagüzel et al., 2024). Due to its premium status, it plays a vital role for many players in the European floral industry, from growers to retailers and many others along the supply chain (EU to fund Pelargonium promotional campaign, 2024. Retrieved from https://www.floraldaily.com/.../)

In Romania (Toma et al., 2012; 2021; Mihuț, 2021), as in most other European countries (Faust et al., 2016; Havardi-Burger et al., 2020), the pelargonium is one of the most popular flowers, used for planting in pots or in the ground, in protected areas or in ornamental gardens (Gabriel, 2015; Moldovan et al., 2023).

Thrips are one of the main insects affecting the quality of flowers, in this case pelargomium flowers, both in the garden and in greenhouses (Lewis, 1997; Atakan, 2019; Jandricic et al., 2024). Therefore, knowledge of the population densities, temporal and spatial distribution of these species in different cultivars of pelargonium is essential for the selection of the most effective control strategies.

The number of thrips species varies greatly on a global scale: Lewis (1997) estimated 8000 thrips species, of which 5500 are pests; Tang et al. (2023), cited by Vîrteiu et al. (2024), estimated 7700; and Mound (2002), cited by Pizzol et al. (2014), estimated 10,000; one thing common to all authors in the literature is

that only about 1% of all recently recorded species are considered pests (Morse & Hoodle 2006; Zvaríková et al., 2020).

The *Thripidae* family are the most serious thrips pests of ornamental plants (Funderburk, 2007; Mirab-Balou et al., 2015; Atakan, 2023).

Due to ongoing taxonomic revisions and new species discoveries, it is difficult to determine the exact number of thrips species in Europe. However, over 300 species of thrips are estimated to occur in Europe (*Thrips*, Order: *Thysanoptera*, 2024. Retrieved from https://www.amentsoc.org/insects/.../), of which 91 species were recorded in Romania according to Knechtel (1951). By 2002, however, Vasiliu-Oromulu reported that the number had exceeded 150, indicating significant progress in taxonomic research and identifying species over the decades. This increase is likely to be due to: improvements in sampling methods - better traps and identification techniques; changes in habitat and climate - which may have affected thrips diversity; introduction of new species - possibly through trade and horticulture (Marullo et al., 2021). Little is known about the population dynamics and management of *Thripidae* in greenhouse grown pelargoniums in Romania. Nevertheless, in the last 15 years of research, a significant number of thrips species have been mentioned in ornamental plants according to Bărbuceanu & Vasiliu-Oromulu (2012) and Vîrteiu et al. (2015; 2022; 2024): *Frankliniella intonsa* (Hinds), *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Trybom), *Frankliniella fusca* (Hinds), *Frankliniella fasciatus* (Linnaeus), *Heliothrips haemorrhoidalis* (Bouché), *Scirtothrips dorsalis* (Hood), *Thrips physapus* (Linnaeus), *Thrips palmi* (Karny) and *Thrips tabaci* (Lindeman).

Frankliniella (*Thripidae*), one of the most widespread genus of thrips, attacks the inflated buds and open flowers of all types of ornamental plants, causing necrosis, cell collapse, deformation, discolouration, necrotic spots with a silvery sheen (Childers & Achor, 1991; Childers, 1997; Shipp et al., 2000), thus reducing the ornamental value of these plants.

The cosmopolitan species *Frankliniella occidentalis* (Pergande) (*Thysanoptera: Thripidae*) has the greatest economic impact on pelargoniums, but species density-damage relationships vary.

The aim of this work was to evaluate the efficacy of biopesticides in controlling thrips species in indoor pelargoniums to maintain high ornamental value for marketing in 2024.

MATERIALS AND METHODS

Survey

The research was carried out under greenhouse conditions in Dudești Noi, located in the north-central part of the Timiș County (45° 50'16" N, 21° 06'02" E, 99 m above sea level), with an area of 5,039.5 ha, mainly used for agricultural purposes (3550.7 ha) and for growing vegetables and flowers in greenhouses (8.63 ha). The greenhouse consists of an air-inflated, double-layered polyethylene structure with an area of 300 square m, where pelargonium are grown on tabletops in small, individual pots, covering a total of 90 m^2.

Plant material and indoor bioassays

The efficacy of bioinsecticide applications approved by the Ministry of Agriculture and Rural Development - Romania was evaluated for thrips control in pelargonium crops in western Romania during spring 2024. The evaluated bioinsecticides are listed in Table 1.

Table 1. Bioinsecticides evaluated for thrips management in *Pelargomium* crops in greeanhouse, Dudești Noi, 2024

Trade nume	Active ingredients	Manufacturer	Rates applied/ 5 m^2
Laser™ 240 SC	*spinosad*	Corteva Agriscience	4.5 ml
Neemex	Neem Oil (*Azadirachta indica*)	B.H.S. Bio Innovation	9.0 ml
Diatom trips	Diatomaceous earth	B.H. Homevo	45 g
Azor	potassium soap	Quelagrow Iberica	18 ml
Exalt	spinetoram	Corteva Agriscience	18 ml

The plants used in the experiment were obtained from seeds. Sowing was performed on January 10, 2024. Immediately after sowing, spray watering with Previcur Energy was performed and the substrate was covered with plastic film (stretch) until emergence. After 8 days, when young seedlings emerged, another watering was performed, also with Previcur Energy fungicide.

Transplanting into individual pots was carried out when the plants had 1-2 true leaves. In order to obtain a vigorous crop, with a high aesthetic and ornamental value, respectively a high commercial value, a series of fertilizers were applied from transplanting to flowering as follows: - Algaren Twin, MagicP Star at transplanting; - King Life 12 - 48 - 8 + Mg, Calfomyth, Drin, Foliacon 22 and King Life 30 - 10 - 10 + Mg at 10, respectively 20 days after the transplanting; - King Life 20 - 20 - 20 and Calfomyth at bud burst; King Life 8 - 5 - 40 + Mg, Algaren Twin and Calfomyth at flowering.

The varieties used in the experiment were as follows *Pelargonium peltatum* x *zonale* 'Anthony' and *Pelargonium peltatum* 'Blue Wonder'.

There were 6 treatments in the trial: 4 bioinsecticides x 1 chemical insecticide plus untreated control (see Table 1), arranged in a randomised complete block design with 3 replications (18 plots in total). Treatment applications began when thrips densities reached 20 adults/trap and the application date was 13 July 2024. The bioassays were evaluated at 24, 72 hours and 7 and 14 days. All insecticide treatments were applied using a pressurized backpack sprayer. A 1.2 m long boom fitted with four twin-flat fan nozzles (Peis HM 4301, Spraying Systems) was used at a walking speed of 1.2 m/s calibrated to deliver 6-8 l/min.

Thrips sampling and identification

The surveys were carried out from the early spring (February) to the summer (July) of 2024, with sampling taking place at intervals between 7 and 10 days. Double-sided sticky traps of different colours (blue and yellow) were used to collect thrips individuals from each pelargomium variety.

The sampling methods are in line with those used by Vîrteiu et al., 2018; Muntean & Grozea, 2021; Vîrteiu et al., 2021; 2022; 2024. Thus, double-sided sticky traps /plot (two/colour) were used, placed at 0.5 m apart, in the middle of each plot. A plot has a perimeter of 5 m^2 and consists of 120 pelargonium plants (Figure 1).

Each month the traps were replaced and taken to the laboratory for preparation and identification. In the laboratory samples were kept in AGA solution for one day for body clearing. Thrips were kept in a 10% solution of NaOH until the specimens became light in colour.

CMCP 10 High viscosity mountant medium has been used to slide mounted thrips.

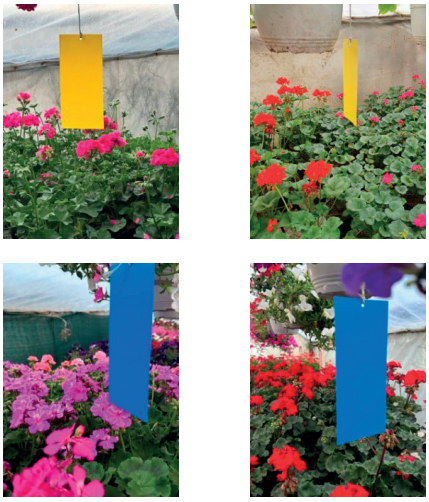


Figure 1. Double-sided sticky traps that were used in the experiment

Slide-mounted adult thrips were identified by using the keys given by zur Strassen (2003); Mound & Marullo (1996); Mound & Kibby (1998).

Statistical analysis

Statistical analyses were performed in R- 4.4.2 for Windows (83 megabyte, 64 bit). R is available as Free Software under the terms of the Free Software Foundation's GNU General Public License in source code form. Average values, standard deviations, coefficients of variation were calculated and the Tukey HSD and Duncan's multiple range tests were applied to determine significant differences between plots. The Henderson - Tilton test was used to calculate the efficacy coefficient for reducing the thrips population.

$$E\% = \left(1 - \frac{n \text{ in Co before treatment} * n \text{ in T after treatment}}{n \text{ in Co after treatment} * n \text{ in T before treatment}}\right) * 100$$

where:
n = thrips population,
T = treated,
Co = control

RESULTS AND DISCUSSIONS

The economic importance of thrips in the past ten years, both in terms of direct, mostly qualitative (Trdan et al., 2008) and indirect, through viroid and phytoplasma transmission (Mound, 1997; Jenser et al., 2003) losses on ornamentals in protected areas, especially on pelargoniums, has necessitated continuous adaptation of IMPs in the majority of cases. This, combined with the need to adapt to an ever-changing phytopharmaceutical market, with the constant withdrawal of some pesticide active ingredients, encouraged us to look for alternative solutions to control thrips in pelargonium crops.

The occurrence of thrips was observed from 8 February to 13 May 2024 on all the pelargonium varieties (Table 2). Data analysis revealed a rich thripsofauna in Dudeștii Noi greenhouse, consisting of a total of 635 individuals of five thrips species, namely: *Frankliniella occidentalis, Frankliniella intonsa, Thrips tabaci, Thrips major and Scirtothrips dorsalis*. *Scirtothrips dorsalis* was one of the new species introduced to our country and collected from the pelargonium crop in 2024. The mean abundance of thrips on the pelargonium varieties Anthony and Blue Wonder ranged from 1.29 to 26.85/trap and 1.29 to 13.5/trap, respectively.

Table 2. Abundance of thrips on colored sticky traps on 2 *Pelargonium* varieties from February to May 2024 at Dudești Noi (Timiș County), Romania

Observation date	Abundance of thrips (number/ sticky trap/varieties)/									
	Frankliniella occidentalis		*Frankliniella intonsa*		*Thrips tabaci*		*Thrips major*		*Scirtothrips dorsalis*	
	*A	BW **	*A	BW **	*A	BW **	*A	BW **	*A	BW **
08-Feb	8^{ab}	0^{a}	5^{a}	0^{a}	11^{a}	3^{a}	0^{a}	0^{a}	1^{a}	0^{a}
23-Feb	13^{ab}	2^{a}	5^{a}	1^{a}	8^{a}	9^{a}	0^{a}	1^{a}	0^{a}	1^{a}
10-Mar	17^{ab}	11^{a}	17^{a}	0^{a}	7^{a}	11^{a}	0^{a}	0^{a}	4^{a}	0^{a}
27-Mar	8^{ab}	11^{a}	9^{a}	12^{a}	1^{a}	7^{a}	2^{a}	1^{a}	2^{a}	0^{a}
11-Apr	39^{ab}	17^{a}	10^{a}	17^{a}	19^{a}	21^{a}	2^{a}	5^{a}	6^{a}	4^{a}
29-Apr	44^{ab}	21^{a}	8^{a}	11^{a}	31^{a}	35^{a}	5^{a}	2^{a}	2^{a}	4^{a}
13-May	59^{ab}	19^{a}	6^{a}	7^{a}	44^{a}	8^{a}	0^{a}	0^{a}	1^{a}	0^{a}

*A - Anthony variety; BW** - Blue Wonder variety**;** Values in columns followed by the same letter(s) are not significantly different to each other according to Tukey HSD tests at $p \leq 0.05$.

Frankliniella occidentalis was by far the most abundant species with 188 specimens collected and an average of 26.85 specimens per trap for the Anthony variety, while *Thrips tabaci* was the second most abundant species (n= 121; $\bar{x}$= 17.29). The most abundant species on the Blue Wonder variety was *Thrips tabaci* with 94 specimens collected and an average of 13.43 specimens per trap. The least collected species was *Thrips major*, with an average of 1.29 specimens per trap for both species studied. In the case of *Frankliniella intonsa*, we can highlight the very high abundance in the pelargonium flower in comparison with the other 3 species of thrips, which preferred to feed on the terminal flower buds and less on the flowers.

Covaci et al. (2012) and Vîrteiu et al. (2024) considered *F. occidentalis* as the main pest of ornamental greenhouse crops in the north-western part of Romania. This species is particularly attracted to inflorescences (van Dijken et al. 1995), and pelargoniums are plants that continue to flower year-round; these are some of the most important conditions that provide a constant food source, leading to an exponential increase in the populations of this species.

ANOVA test for differences in abundance between thrips species ($F = 7.07$; $df = 9$; $p = 0.027 < 0.05$) confirms significant differences between number of thrips species/pelargonium variety collected during monitoring period (Table 3).

Table 3. One-way analysis of variance (ANOVA) evaluating the abundance of thrips species in relation to different variables of greenhouse pelargonium crop monitoring

Source	df	F	*p*	Sig.
pelargonium varieties	9	7.07	**0.027**	<0.05
phenological periods	14	4.48	**0.035**	<0.05
feeding regime	9	0.48	0.750	

Bold p-values ($p<0.05$) indicate a significant positive correlation; for $p \geq 0.05$ no significant correlations were observed.

There was a gradual seasonal increase in the number of thrips up to a peak period for each species (Figure 2). Depending on the developmental cycle of each species, preferred feeding sites and vegetative organs, the peak periods of each species were variable.

In this study we provided evidence that the distribution of thrips species in the foliage and inflorescences of pelargonium is not uniform.

There was a close interaction between crop phenological periods and thrips species, with the proportion of thrips species increasing significantly with the flowering period ($F = 4.48$, $df = 14$, $p = 0.035 < 0.05$) (Table 3).

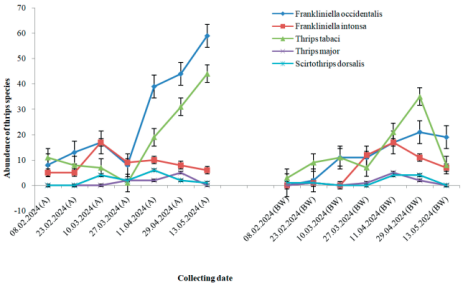


Figure 2. Abundence and seasonal density of thrips species on pelargonium plants (A = Anthony variety; BW = Blue Wonder variety)

In the monitored greenhouse, a higher distribution of thrips species was observed in the inflorescences than in the foliage (Figure 3).

Our general results are in agreement with those of Betanzos et al. (1999) and Palomo et al. (2015), who mentioned that the highest thrips populations were during flowering and in the warmer periods.

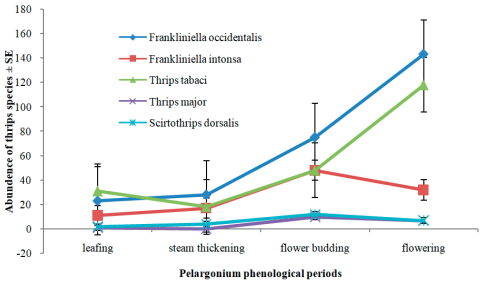


Figure 3. Thrips abundance related to pelargonium phenological periods

Following Healey et al.'s (2017) research, it highlights that in horticultural crops, increases in thrips abundance are linked to crop phenology, with densities increasing as crops mature. Similarities were observed in this study, with population establishment and increased abundance coinciding with successive crop phenological periods: leafing, budding and flowering.

Similar results were obtained by Trdan (2003), who also found that the main factor affecting thrips populations was the plant's phenological stage.

Our results on feeding regime provide experimental evidence for the constant presence of extremely polyphagous species, such as *Frankliniella occidentalis, Frankliniella intonsa* and *Thrips tabaci*. We also observed that pelargoniums strongly facilitated the population growth of *Thrips major* and *Scirtothrips dorsalis*, species with an oligophagous feeding regime, which was particularly rapid from the beginning of budding, whereas the effect of oligophagous species on pelargoniums was not significant ($F = 0.48$, $df = 9$, $p = 0.75$) (Table 3).

Due to the cyclical flowering of pelargonium species, but especially the presence of abundant foliage that provides abundant food throughout the growing season, thrips can have continuous and multiple generations in protected areas (Tomassini & Maini, 1995; Hewitt et al., 2015; Vîrteiu et al., 2024). The density of these pests is also determined by and closely correlated with the availability of other flowering plants in the study area.

Considering that, in addition to *Pelargonium, Petunia, Dahlia, Gazania*, etc. are the preferred host plants of the pests studied, it is difficult to establish the population dynamics.

Flowering ornamentals plants are highly attractive to thrips, allowing rapid population growth (Rhainds et al., 2003, 2005). Thrips feeding activities lead to early flower senescence and leaf abscission (van Dijken et al., 1995), which reduces plant nutrient quality for thrips feeding and reproduction (Nyasani et al., 2013). This suggests that control may need to be timed to coincide with these early stages of growth to reduce the increase in abundance that follows these periods of establishment of thrips populations (Healey et al., 2017).

The results obtained have contributed to the sustainable reduction of thrips populations by identifying the optimum time to apply appropriate thrips control methods. This approach supports an increase in the quality and ornamental value of the plants studied. Data analysis indicates that mid-May is the optimal time to apply treatments, as this is when the highest population levels were recorded for all species analysed.

Due to the very high incidence observed in the samples, and primarily to establish an effective management practice by reducing pest pressure, various botanicals, biological and chemical products were tested in 2024. This approach aims to maintain the high quality of these ornamental flowering plants for commercialization as quickly as possible.

Table 4 shows the response of different insecticides and bio-insecticides on thrips adult population, revealing significant variation in all treatments.

Coefficient of variation (CV %) ranged from 23.65% to 49.34%, indicating relatively low within-treatment variability, suggesting that the data are consistent.

Table 4. Assessing the response of bio-insecticides and chemical insecticides to mean thrips adult populations in pelargonium crops, 2024

Treatments	Mean no. adults/colored sticky traps							
	after 24 hours	E%	after 48 hours	E%	after 7 days	E%	after 14 days	E%
T1 = untreated control	39[a]	-	35[a]	-	41[a]	-	44[a]	-
T2 = Laser 240 SC/ *spinosad*	32[b]	34.69%	11[b]	77.55%	26[b]	46.94%	41[b]	16.33%
T3 = Neemex/ neem oil (*Azadirachta indica*)	40[b]	21.57%	19[b]	62.75%	25[b]	50.98%	47[b]	7.84%
T4 = Diatom trips/ diatomaceous earth	31[c]	20.51%	29[c]	25.64%	33[c]	15.38%	34[c]	12.82%
T5 = Azor/ potassium soap	20[c]	25.93%	15[c]	44.44%	19[c]	29.63%	24[c]	11.11%
T6 = Exalt/spinetoram	17[d]	60.47%	8[d]	81.4%	21[d]	51.16%	27[d]	37.21%
Mean	29.83		19.5		27.5		26.17	
CV%	29.18		49.34		27.19		23.65	
Sig	*		*		*		-	

*Values in columns followed by the same letter(s) are not significantly different to each other according to to Duncan's multiple rangetests at $p \leq 0.05$, CV- coefficient of variation, Sig- significance level, *Significant at $p < 0.05$, E% - efficiency coefficient

Chemical insecticides such as spinetoram were more effective than the bio-insecticides used, but the latter were significantly more effective than untreated controls at all four time points: 24, 48 hours, 7 and 4 days post treatment.

After 24 hours, in the analysis including the untreated control, the insecticides (bio and chemical) significantly affected the mean thrips densities ($F = 5.76$; $df = 11$, $p = 0.037 < 0.05$). The efficacy of spinetoram was higher (60.47% reduction) compared to the bio-insecticide, where the highest reduction rate was obtained with spinosad (34.69%). Potassium soap, neem oil and diatomaceous earth showed reductions between 20.51 and 25.93%. A significant difference between the efficacy of the chemical and biological products was observed after 24 hours post treatment.

Direct exposure to the toxicity of chemical and biological insecticides resulted in a significant reduction in adult thrips density 48 h after treatment ($F = 16.97$; $df = 11$; $p = 0.0021 <$

0.05). Among the chemical insecticides, spinetoram was found to cause the highest reduction of all thrips species compared to the control, reaching 81.4%. A similar level of efficacy was also shown by the bio-insecticides, both spinosad and neem oil, with a coefficient of efficacy of 77.55% and 62.75%, respectively. Average thrips numbers were reduced to moderate levels by potassium soap and diatomaceous earth.

7 DAT, all bio and chemical insecticides tested significantly reduced thrips infestation compared to the control treatment ($F = 9.44$, $df = 11$, $p = 0.012 < 0.05$). In general, the greatest reduction in thrips population by chemical insecticides was achieved by spinetoram (51.16%), while the best bioinsecticide was neem oil extract (50.98%), followed by spinosad (46.94%).

At 14 DAT, no significant differences ($F = 1.70$, $df = 11$, $p = 0.220$) were observed in the mean number of thrips collected with coloured sticky traps in pelargonium plants. Efficacy varies between 37.21 and 7.84%, with chemical spinetoram achieving the best results (37.21%), followed by spinosad.

The untreated control exhibited a gradual decrease in thrips populations over time, likely due to natural mortality or environmental factors. Treated groups generally showed an initial increase in mortality within 24-48 hours, with varying levels of persistence up to 14 days.

The highest relative differences compared to the control were observed in spinetoram (Exalt), spinosad (Laser 240 SC) and neem oil (Neemex) treatments, suggesting a strong initial impact on thrips populations.

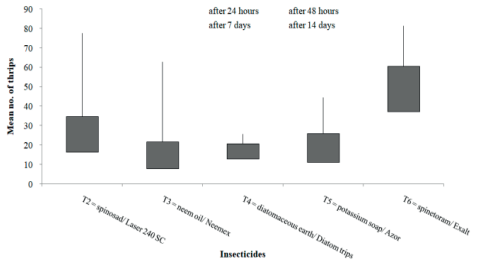


Figure 4. Efficacy of bio and chemical insecticides against thripspopulation, greenhouse pelargonium crops, 2024

The treatments with diatomaceous earth and potassium soap showed a more moderate effect, with reduction coefficients that were closer to those of the control.

These findings indicate that while some treatments significantly reduce thrips populations, further studies with larger sample sizes are required to confirm statistical significance and optimize treatment efficiency.

The results obtained by Lahiri et al. (2024) show us the need to increase the reliance on biopesticides for thrips management, while at the same time highlighting the evidence that plant-based biopesticides can act as valuable rotational products with conventional insecticides for thrips management. In this study, 5 conventional and biological insecticides were tested under greenhouse conditions for their efficacy on thrips populations in pelargoniums. Our observations consistently showed that chemical products showed the highest efficacy under direct contact conditions. These data agree with those of Ataide et al. (2024) who identified 3 conventional and 2 biorational insecticides as most effective against thrips under laboratory conditions. However, research by Kuar et al. (2023) on *Scirtothrips doralis* has shown that this species has acquired resistance to chemical insecticides, including spinetram, with high thrips densities similar to those in untreated control plots. Our study shows that two bioinsecticides, spinosad and neem oil, are most promising for effective thrips control.

In support of our findings, Manideep et al. (2023) showed that spinosad was first in the order of efficacy, reducing 80% of thrips infestations on chrysanthemum plants in the field. Also, Hassan et al. (2024), testing a variety of insecticides in the management of *Thrips tabaci*, certified spinosad with a greater efficacy (94.5%) compared to spinetoram (89.7%). The same authors mention neem oil with 64.1% efficacy, very close to that obtained by us with the same active ingredient (62.75%) 48 hours after application. Monteon-Ojeda et al. (2021) found that three consecutive applications of neem extract were sufficient to reduce *Frankliniella occidentalis* infestation by more than 75%, and spinosad was the best treatment, achieving 90% efficacy with only two applications, positioning it as the best

biological option for thrips control. Sahoo & Tripathy (2020), however, recommend using spinosad together with neem oil.

CONCLUSIONS

The overall conclusion of this greenhouse study is that the spinosad-based bioinsecticide can provide reliable protection against thrips species in pelargonium plants, which is of great importance to ornamental growers who continue to struggle with thrips populations that have developed resistance over time to a very wide range of chemicals. Although the performance of neem oil under protected conditions varies, measures can be taken to use this product in parallel with spinosad for the most effective control of thrips in both floricultural and horticultural greenhouses. From a practical point of view and depending on the results obtained, we recommend alternating the spirotoram-based chemical with the single application spinosad/ neem oil combination products.

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ANATOMICAL AND BIOCHEMICAL RESEARCH ON THE SPECIES *ARTEMISIA ABSINTHIUM* L. (ASTERACEAE) FROM THE SOUTHERN PART OF ROMANIA

Monica Luminița BADEA, Vasilica LUCHIAN, Liliana BĂDULESCU, Elena DELIAN, Elena SĂVULESCU

Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: monica.badea@horticultura-bucuresti.ro

Abstract

Artemisia absinthium L. (Wormwood) is a medicinal plant known for its antibacterial, antioxidant, anti-inflammatory, antifungal, antimalarial, and choleretic properties. The essential oil, extracted through hydrodistillation, was analyzed using GC-MS. The main chemical compounds identified in the essential oil were linalyl isovalerate (16.65%), sabinene (8.90%), myrcene (8.85%), geranyl isobutyrate (6.97%), and β-cymene (6.92%) in the stems; sabinene (23.67%), myrcene (20.53%), β-cymene (9.74%), and α-phellandrene (9.23%) in the leaves; and chrysanthenone (5.48%), sabinene (11.18%), beta-pinene (15.53%), and linalool (17.97%) in the flowers. Anatomical analysis was performed on cross-sections of the stems and leaf lamina. Transverse sections of the stems and leaves were examined under an optical microscope. The A. absinthium stems and leaves are covered with dense T-shaped glandular and non-glandular trichomes, on both sides, the most vigorous being those on the stem and petioles.

Key words: *Artemisia, essential oil, chromatography, glandular trichomes.*

INTRODUCTION

Artemisia absinthium L. (Wormwood) is a medicinal and aromatic bitter herb (Nguyen & Németh, 2016), belonging to the Asteraceae family, the Artemisia genus, including over 500 species (Hayat et al., 2009; Ivănescu et al., 2021; Tojić & Rančić, 2023). It is a perennial plant native to the temperate regions of Asia, Europe, North Africa, Canada and the United States (Bhat et al., 2019). Extracts from these plants contain several classes of secondary metabolites such as: flavonoids, glycosides, coumarins, polyacetylenes, sterols and terpenoids (Tan et al. 1998). The secondary metabolites of Artemisia species are used in the health field but also in food and cosmetics (Ivănescu et al., 2021).

The main secondary metabolites that are responsible for the aromatic odor are found in the leaves and flowers (Bordean et al., 2020). Studies have shown that *Artemisia absinthium* L. plants have antimicrobial, anti-inflammatory, antioxidant, antifungal, antibacterial, antimalarial, anti-tumour choleretic and gastrointestinal effects (Bora & Sharma, 2010; Ebrahimzadeh et al, 2010; Hussain et al., 2017; Goud & Swamy, 2015; Bhat et al., 2019; Batiha et al., 2020; Szopa et al., 2020; Malla et al., 2023). Also, the chemical compounds present in the volatile oil from Artemisia plants show parasiticidal, insecticidal and phytotoxic properties, being recommended as possible biological control agents against plant pests (Ivănescu et al., 2021).

This species is well known for its use in the production of the absinthe drink, being restricted in many countries due to neurotoxicity (Sharifi-Rad et al., 2022). The species of this genus show great therapeutic potential being used against various health problems around the world (Hussain, 2020).

The anatomical elements of species are very important in the classification and correct identification of plants (Hussain et al., 2019; Hussain, 2020).

Studies related to the anatomy of this species were conducted by Konowalik & Kreitschitz (2012), Ivanescu et al., (2015), Ivashchenko et al. (2017) and Janaćković et al. (2019).

The objective of this study is to highlight the importance of *Artemisia absinthium* L. by analyzing the chemical composition of the

essential oil extracted from the stems, leaves, and flowers, as well as analyzing the plants from an anatomical perspective.

MATERIALS AND METHODS

Biological material

For the anatomical analysis, mature stems and leaves (leaf blade and petiole) of the species *Artemisia absinthium* L. were used. Regarding the essential oil analysis, it was extracted from fresh plants (stems, leaves, flowers).

The plants were collected from the southern part of Romania, Teleorman County.

Anatomical characterization

The microscopic examination was performed on transverse sections obtained from leaves and stems, analyzing fresh preparations treated with chloral hydrate and stained with carmine alum and methyl green (Luchian et al., 2018), according to the classical method.

The study was carried out in the Botany Laboratory – Faculty of Horticulture Bucharest. The photos have been take using an Optika optical microscope with different objectives, and a Motorola digital camera.

Volatile oil analysis

The essential oil was extracted and analysed within the Faculty of Horticulture Bucharest. Some herbal parts (stems, leaves and flowers) were subjected to hydro distillation for 3h in a fresh condition, using a Clevenger apparatus. The components were separated and identified using an Agilent gas chromatograph equipped with a quadrupole mass spectrometer detector.

A DB-5 capillary column (25 m in length, 0.25 mm inner diameter, and 0.25 µm film thickness) was used, with helium as the carrier gas.

The oven temperature was initially set at 60°C and gradually increased to 280°C at a rate of 4°C per minute (Burzo et al., 2008). The identification of essential compounds was performed using the NIST spectral database, with confirmation based on comparison of Kovats retention indices.

RESULTS AND DISSCUTIONS

The composition of the volatile oil

From the analysis of the volatile oil in the *Artemisia absinthium* L. species, a higher number of chemical compounds was observed in the flowers (40) compared to the stems and leaves which had the same number (30). The chemical composition of the essential oil varied according to the organ, as follows: in the stems, the chemical compounds present in greater quantity were: linalyl isovalerate (16.65%), sabinene (8.90%), myrcene (8.85%), geranyl isobutyrate (6.97 %), β-cymene (6.92%), as shown in Figure 1, followed smaller amounts of isopropyl methyl hexenyl acetate (4.45%), neryl butyrate (2.57%), linalol (2.39%), α-phellandrene (2.36%), ocimene (2.14%), α-curcumene (1.83%), camphene (1.68%), nerolidol (1.05%) and terpinen-4-ol (1.01%). Other constituents had a value below 1% (cis β ocimene, eucalyptol, γ terpinene, myrtenol, lavandulol, nerol, hexenyl methyl butyrate, neryl acetate, hexyl methyl butenoate, terpinyl acetate, β elemene, geranyl propionate, isoaromodendron epoxide, α- bisabolol and cedren 13-ol).

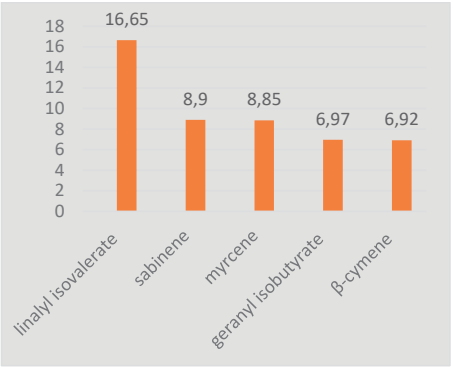


Figure 1. Major chemical compounds of stems, %

In the leaves, the chemical compounds had a greater weight: sabinene (23.67%), myrcene (20.53%), α-phellandrene (9.23 %) and β-cymene (9.74%), as shown in Figure 2, followed smaller amounts of lianlol (4.75%), eucalyptol (2.04%), γ terpinene (1.29%) and myrtenol (1.39 %).

Other constituents had a value below 1% (methyl octene, α-thujene, α-pinene, α-terpinene, cis β ocimene, isopropyl methyl cyclohexen 1-ol, α-terpinolene, hexyl isobutyrate, linalol oxide, lavandulol, dimethyl octadiene diol, α-terpineol, isopulegol, nerol, beta-caryophylene, β-farnesene, β-himachalene, β-selinene, tetra methyl dehydronaphthalene, selinen-4-ol and cis-farnesol).

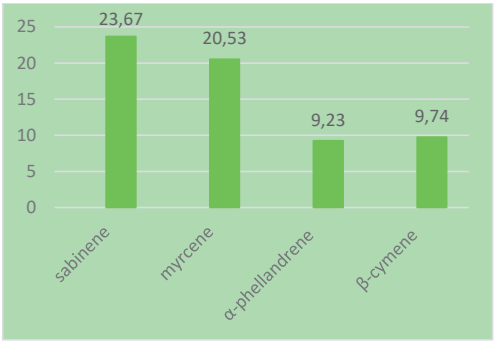


Figure 2. Major chemical compounds of leaves, %

In the essential oil extracted from the flowers, the chemical compounds were in greater quantity: chrysanthenone (5.48%), sabinene (11.18%), β-pinene (15.53%), linalol (17.97%), as shown in Figure 3, followed smaller amounts of tetramethyl-1, 2-dehydronaphalene (4.80%), geranyl butyrate (3.47%), β-himachalene (2.99%), terpinene-4-ol (2.87%), α-phellandrene (2.48 %), selinen 4-ol (2.11%) , linalyl isovalerate (2.31%), r-muurolene (1.58%), γ-terpinene (1.43%), β-cymene (1.26%), β-phellandrene (1.12%) and nuciferol (1.04 %).

Other constituents had a value below 1% (α-thujene, α-pinene, camphene, octanol, α-terpinene, β-thujene, trans-β-ocimene, α-terpinolene, cis p-terpineol, lavandulol, cis-β-ocimene, nerol, neryl acetate, geranyl isobutyrate, β-caryophyllene, β farnesene, β selinene, nerolidol, epiglobulol, caryophyllene oxide, chamazulene, methyl propenyl tetrahydronaphthalene 2-ol and cis-lanceol).

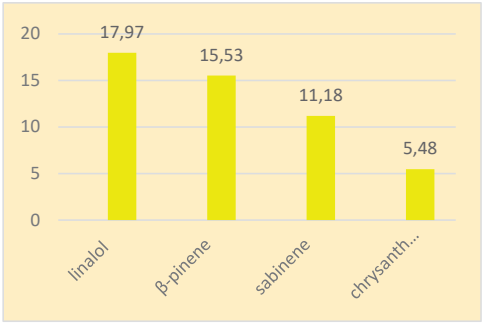


Figure 3. Major chemical compounds of flowers, %

Sabinene was present in greater quantity in the composition of the essential oil extracted from leaves (23.67%), followed by flowers (11.18%) and stems (8.90%). Regarding the chemical compound β-cymene, a larger amount was observed in the stems and leaves (6.92-9.23%), and a much smaller amount in the flowers (1.26%).

Linalol was present in greater quantity in the essential oil extracted from the flowers (17.97%), and in the volatile oil extracted ace from the stems and leaves was in smaller quantity (2.39-4.75%).

α-Phelandrene was identified in a larger amount in the composition of the essential oil extracted from the leaves (9.23%), compared to the amount obtained from the volatile oil extracted from the stems and flowers, where the values were approximately equal (2.36-2.48%).

Myrcene was present in greater quantity in the essential oil extracted from the stems and leaves (8.85-20.53%), being absent in the volatile oil extracted from the flowers.

The presence in greater quantity of substances specific to each vegetative organ should be noted. Thus, in the case of the essential oil extracted from the stems, the presence of esters was highlighted: linalyl isovalerate (16.65%), geranyl isobutyrate (6.97%) and isopropyl methyl hexenyl acetate (4.45%), and in the case of the essential oil extract from flowers of monoterpenes: β-pinene (15.53%) and chrysanthenone (5.48%).

Regarding the chemical compounds with a smaller weight, it was observed that there were chemical compounds common to the analyzed organs, such as: α-thujene, α-pinene, α-terpinene, α-terpinolene, α-terpineol, β-caryophyllene, β-selinene, selinen 4-ol (leaves and flowers), eucalyptol and myrtenol in stems and leaves, cis-β-ocimene, lavandulol, terpinene 4-ol and nerol in stems, leaves and flowers. Research by Lopez-Lutz et al., 2008, showed that the volatile oil from *Artemisia absinthium* L. plants from western Canada had a high content of trans-thujone (10.1%), myrcene (10.8%) and trans-sabinyl acetate (26.4%).

The analysis of the volatile oil carried out by Burzo, 2015 on *Artemisia absinthium* L. plants from Romania showed that the majority chemical compounds were the following: β-pinene (41.65%), myrtenyl acetate (12.77%), sabinene (6.68%), α-phellandrene (7.02%) and β-thujone (4.60 %). A high content of sabinene and myrcene (9.2% - 38.9%) were characteristic

of samples from Hungary, Moldova and Scotland (Orav et al., 2006).
The essential oil of *Artemisia absinthium* L. from Lithuania was rich in trans-sabinyl acetate and thujones (Judzentiene et al., 2009).
According to Sharopov et al., 2012, the major chemical compounds present in the essential oil obtained from *Artemisia absinthium* L. plants from three different locations in central Tajikistan were: cis-chrysanthenyl acetate, myrcene, germacrene D, dihydrochamazulene isomer, linalool, linalool acetate and α-phellandrene.
According to the research carried out by Nguyen et al., 2018, a higher content of essential oil was observed in the flowers than in the leaves, regardless of the phenophase (0.31mL/100 g, after flowering stage to 0.91mL/100 g, flowering stage in trans sabinil acetate.
The essential oil content is also influenced by environmental factors, such as light and temperature; the ratio of cis-chrysanthenyl acetate grew from 8.0% to 13.8% (in the "cold chamber" and "warm chamber") with the Spanish plants (Nguyen et al., 2018).

Anatomical characterization

Stem anatomy

Cross-sections have been performed at the internode; fresh slides thereof have been observed under the microscope.
The stem is pubescent, greenish-grey, with a circular contour especially toward the base, where some areas are evidently ridged.
The following areas can be noticed looking from the exterior to the interior of the cross-section: the epidermis, made up of one layer of isodiametric, oval, cuticle-covered cells. It presents dense, simple, T-shaped tector trichomes, bicapitate glandular trichomes and, here and there, stomata (Figure 4 a, b, c).
The cortex is composed of a 7-12 layered collenchyma and a 3-5 layered intercostal parenchyma.
The endodermis is monolayered.
The vascular bundles are collateral, separated from the parenchymatous cells, with 2-12 layers of strongly lignified schlerenchyma above the phloem.
The phloem has small, irregularly-shaped elements. The xylem is made up of 3-8 parallel rows of xylem elements, each row comprising 2-7 vessels.
The central cylinder is sclerified. Small secretory canals can be observed within the cortex. The central part of the cross-section is occupied by the pith, well-developed, formed of parenchyma cells with intercellular spaces. Moreover, the pith also contains small secretory canals (Figure 5 a, b, c, d). Inside the cortex and pith there are secretory canals for essential oils (Figure 6 a, b, c, d,; 6.1. a, b, c).
The longitudinal sections through the stem reveal spiral and punctiform vessels as well as fibers (Figure 7 a, b, c).
The stem epidermis has polygonal, elongated cells, tector and glandular trichomes (Figure 8 a, b, c; 8.1 a, b, c).
The lignified stem presents secondary structure elements, a periderm with overlapping cells, xylem and secondary phloem generated by the cambium.
Anatomy research for this species has also been carried out by Hussain et al., 2019, while Liu et al., 2022, conclude that the morphology and density of the glandular and tector trichomes are highly significant for the diagnosis of *Artemisia* species.

Petiole anatomy

The petiole cross-section has irregular edges and presents the following elements: a monolayered epidermis covered by a thin cuticle, with non-glandular and glandular trichomes resembling those present on the stem and leaves, a parenchymatic tissue including collenchyma, schlerenchyma, secretory canals, and small, medium, and large collateral conducting bundles, made up of xylem and phloem (Figure 9 a, b, c, Figure 10 a, b, c).
The petiole epidermis has polygonal, elongated cells, simple and T-shaped tector trichomes, as well as glandular, bicapitate trichomes (Figure 11 a, b, c).

Leaf blade anatomy

The leaf is equifacial, tomentous on its lower side, with both the upper and lower epidermis having a thin cuticle, with simple and T-shaped non-glandular trichomes, as well as large, silvery-silky glandular trichomes, epidermic cells having their anticlinal walls with a wavy contour, both for the upper (Figure 12 a, b, c, d, e, f) and the lower (Figure 13 a, b, c, d) epidermis.

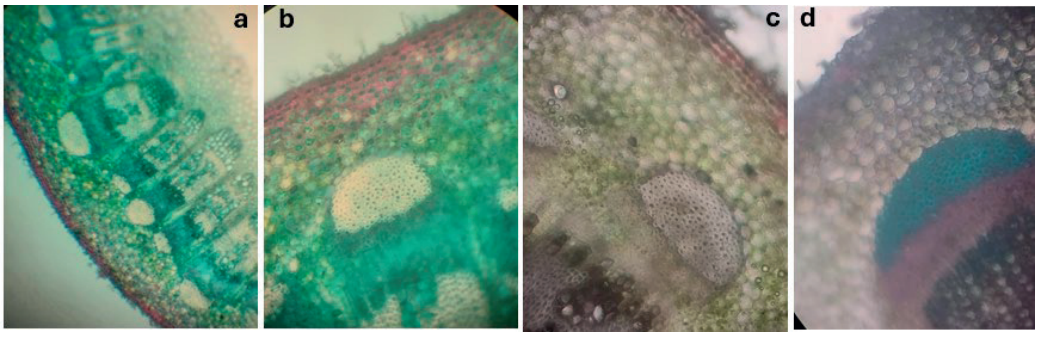


Figure 4. Transversal section of the stem

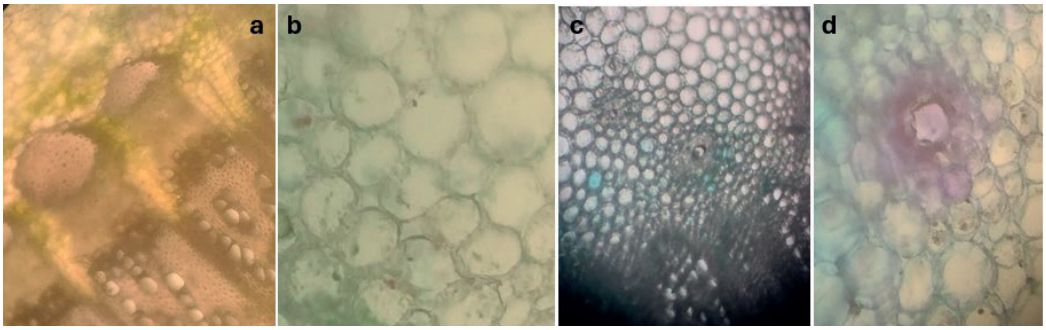


Figure 5. a - Stem - cortex with conductive bundles, b - pith, c, d - secretory canals in the pith - overall view and detail



Figura 6. a, b, c, d -Secretory canals in the cortex - overall view

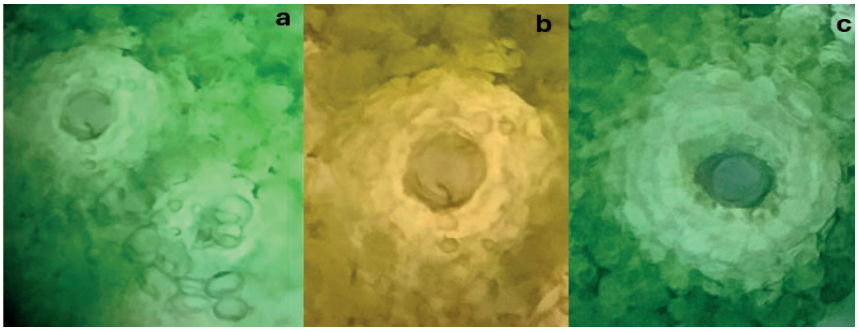


Figure 6.1. a, b, c - Secretory canals in the cortex - b, c - detail

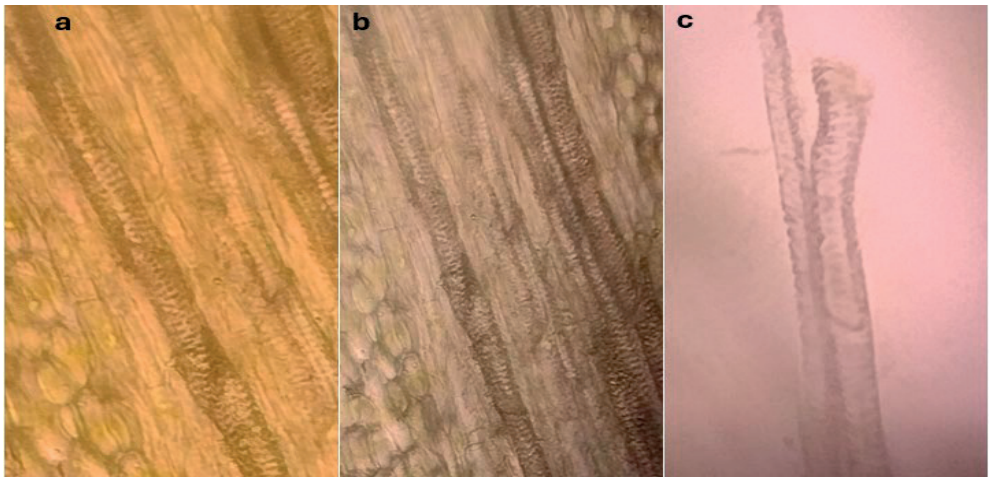


Figure 7. a, b, c, - a,b - Longitudinal section in the stem - punctate conductive vessels and fibers - overall view, c - detailed punctiform vessels

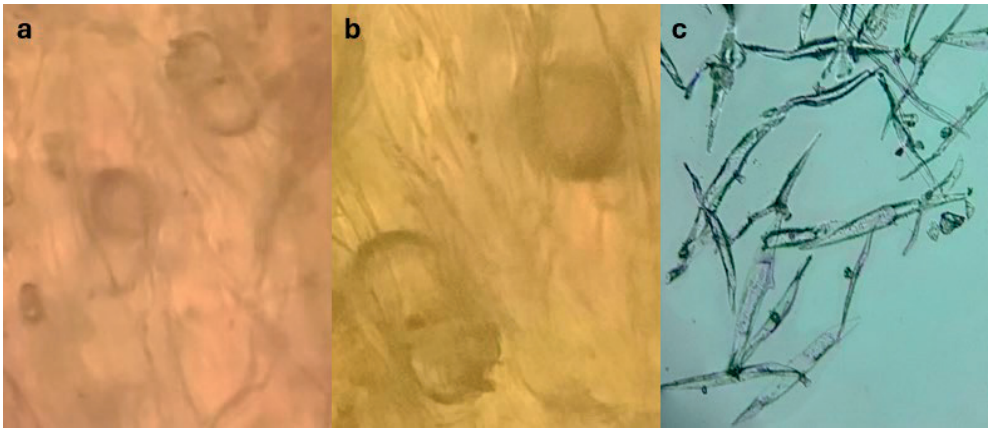


Figure 8. a, b, c - Stem epidermis with tector and glandular trichomes - overall view and detail, c - non-glandular hairs - overall view

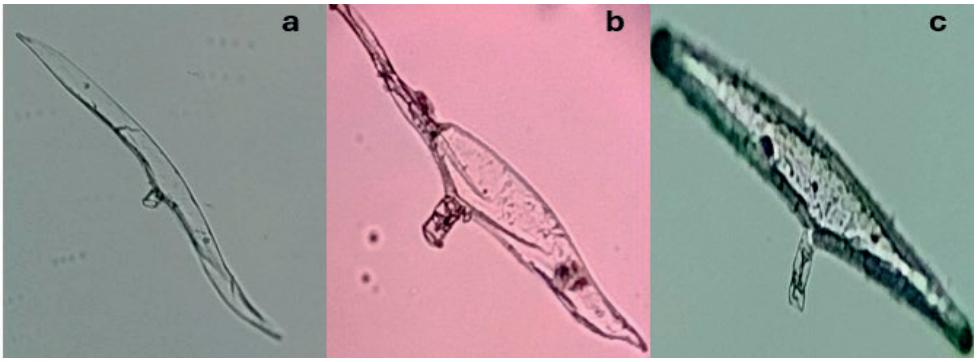


Figure 8.1. a, b, c - Stem epidermis with tector trichomes - overall view and detail, non-glandular hairs - T-shape detail

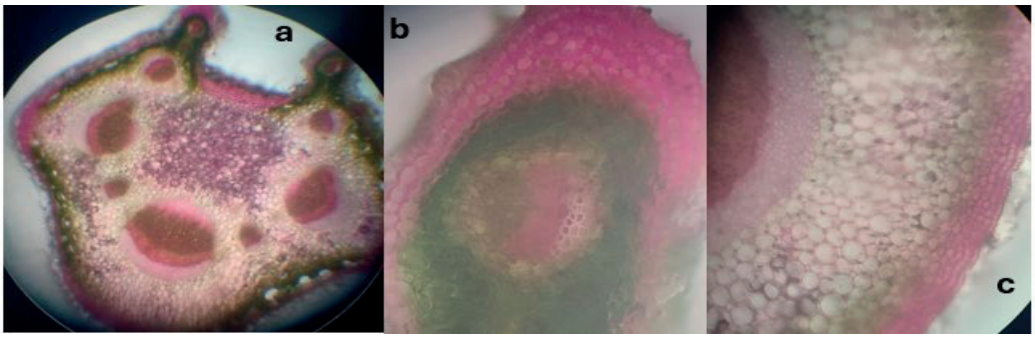


Figure 9. a, b, c - a - Petiole - overall view, b - section margin with conductive bundle, c - detail of the outer zone

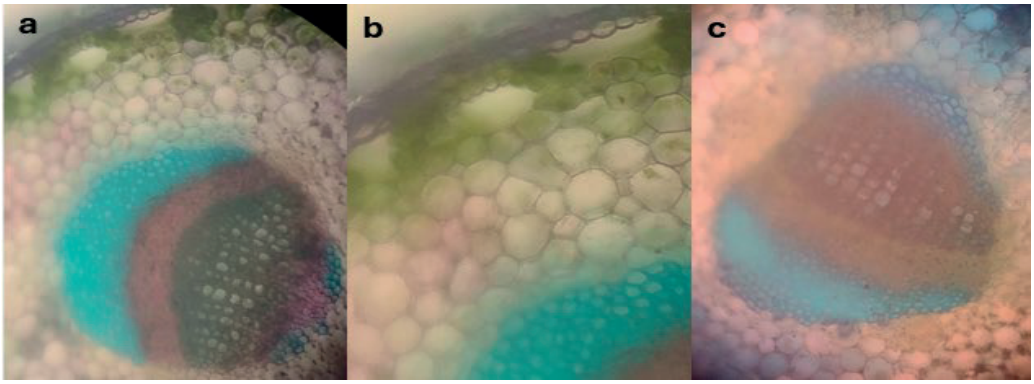


Figure 10. a, b, c - a, b - Petiole with secretory canals and, c - conductive bundle

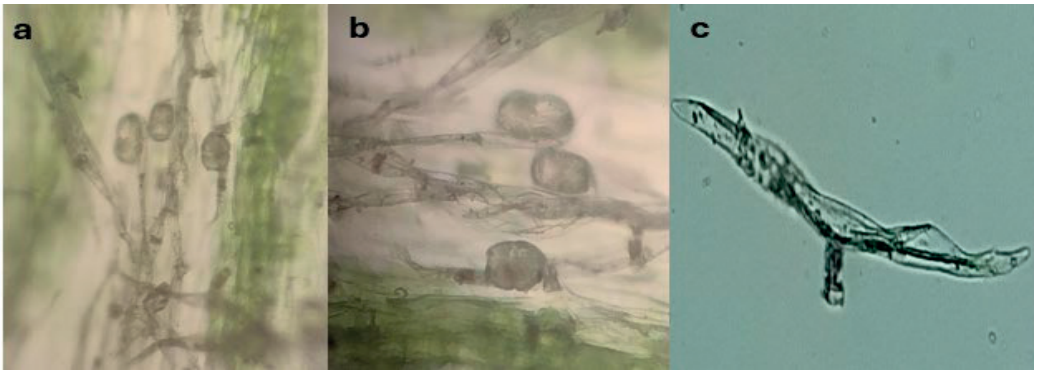


Figure 11. a, b, c - a- Petiole epidermis with tector and glandular trichomes - overall view, b - detail, c - non-glandular trichome in the shape of the letter T - detail

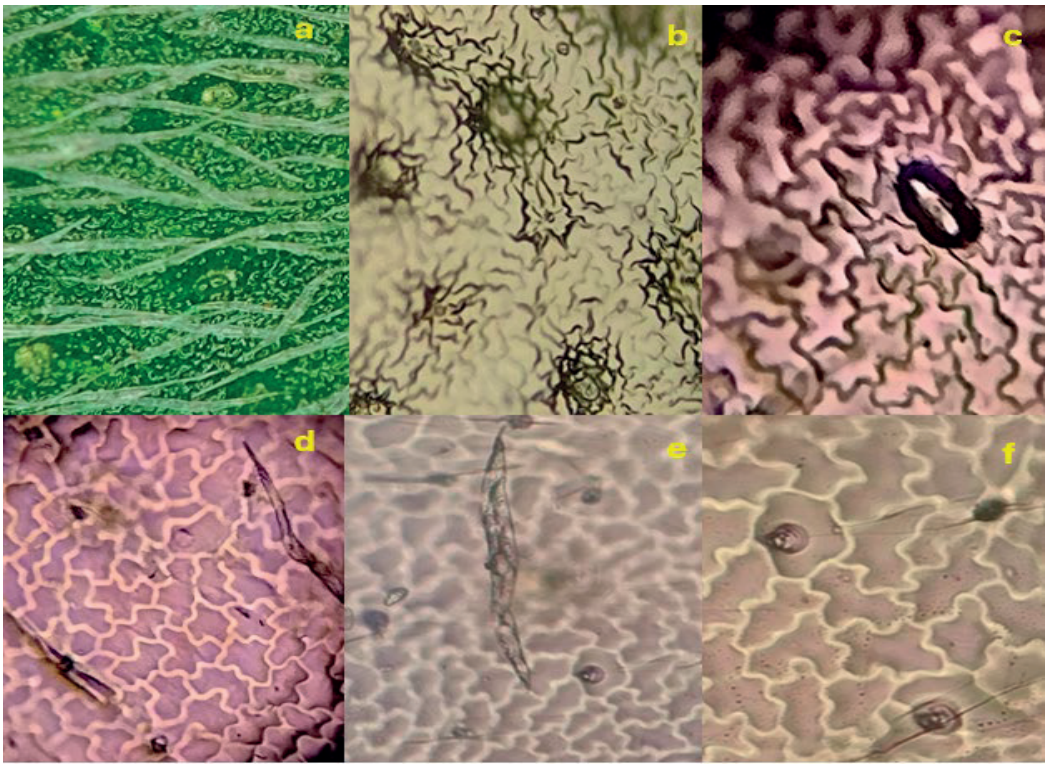


Figure 12. a, b, c, d, e, f -a-Upper epidermis with T-shaped trichomes and glandular trichomes - overall view, b - upper epidermis with glandular trichomes - overall view, c - upper epidermis with glandular trichomes - detail, d, e, f - upper epidermis with T-shaped and simple trichomes

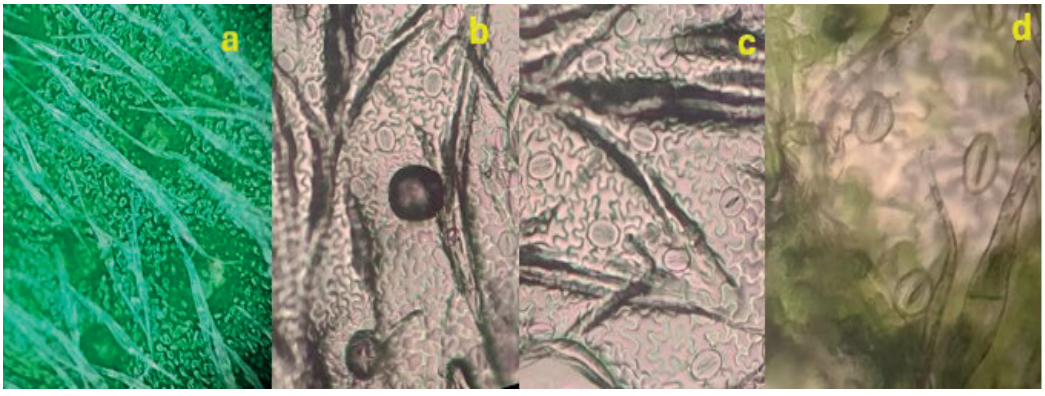


Figure 13. a, b, c, d - Lower epidermis with simple trichomes, T - shaped trichomes, and glandular trichomes, b - lower epidermis with stomata, T - shaped trichomes, simple trichomes, and glandular trichomes, c, d - lower epidermis with stomata, T - shaped trichomes, and simple trichomes

The upper and lower epidermis are both monolayered, with non-glandular simple, pointed or T-shaped trichomes, as well as large, bicapitate, prominent glandular trichomes. Stomata are found only on the abaxial epidermis.

Under the epidermis with T-shaped trichomes (Figure 14 a, b) there is the mesophyll differentiated into a palisadic and a lacunar tissue.

The mono-or bilayered palisadic tissue is positioned adaxially and abaxially, being made up of columnar cells rich in chloroplasts, small-sized lacunar tissue, and collateral conducting bundles in the area of the main and secondary ridges; the xylem is located toward the upper epidermis, with the phloem toward the lower epidermis (Figure 15 a, b, c, Figure 16 a, b, c).

There were many studies indicating that volatile substances of *Artemisia* species are produced and released through glandular trichomes (Zhou et al., 2020). Stem and leaf anatomy research for *Artemisia* species has also been carried out by Osmanlıoğlu et al., (2023) Toma & Rugină (1988).

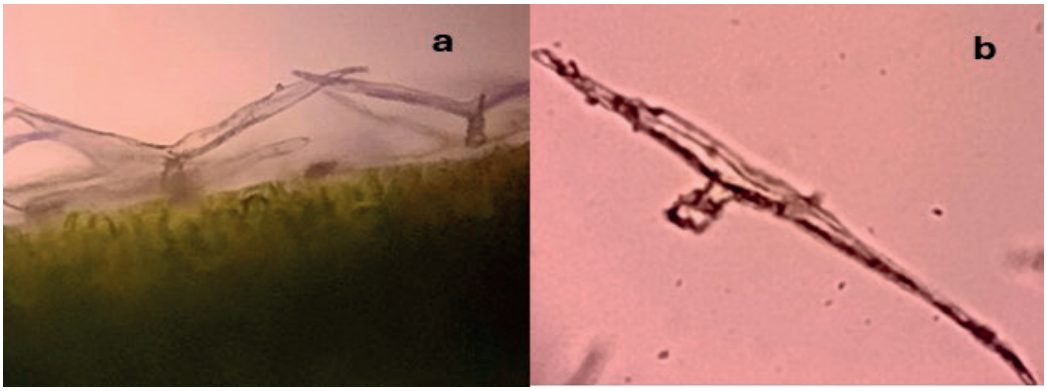


Figure 14. a, b - a - Transverse section in the lamina with epidermis containing T-shaped trichomes - overall view, b - detail of trichome hairs

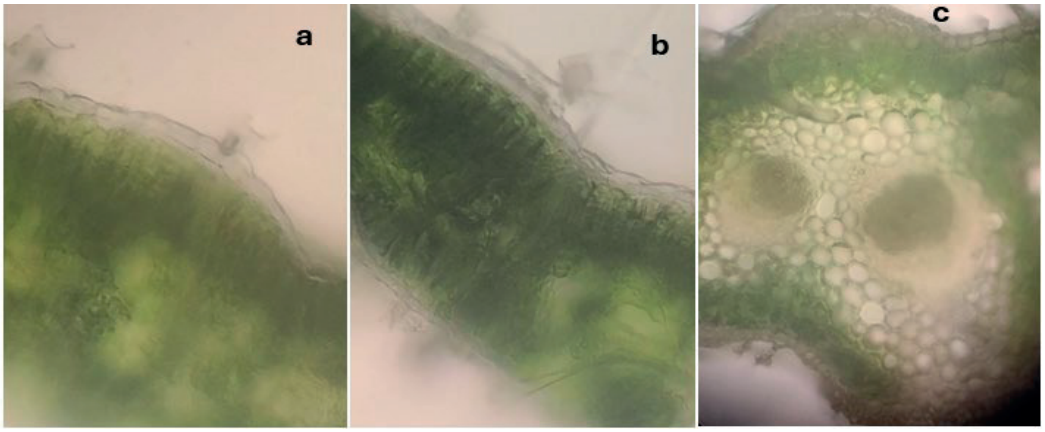
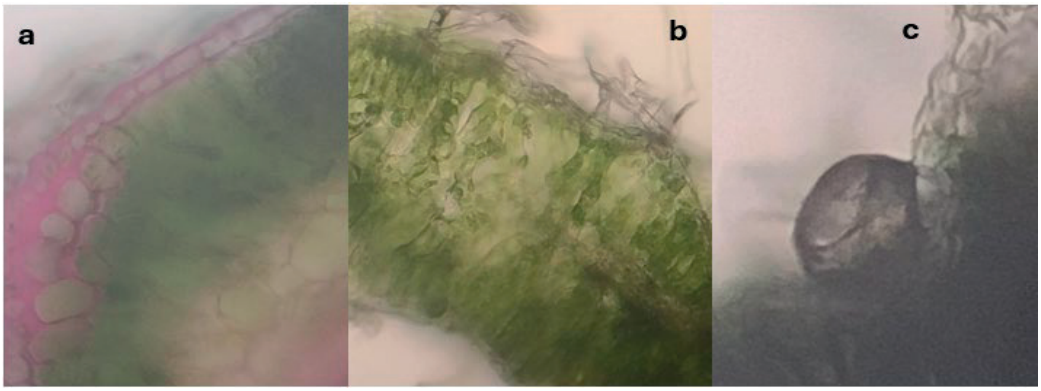


Figure 15. a, b, c - Transverse section in the lamina



Figurae 16. a, b, c - Transverse section in the lamina. a - epidermis, b - mesophyll with palisade tissue beneath both epidermis, c - epidermis with glandular trichomes

CONCLUSIONS

The *Artemisia absinthium* L. species presents a high therapeutic potential with beneficial effects for multiple conditions. The chemical compounds present in greater quantity in *Artemisia absinthium* L. species were: linalyl isovalerate, sabinene, myrcene, geranyl isobutyrate, β-cymene in stems, sabinene, myrcene, α-phellandrene, β-cymene in the leaves and sabinene, beta-pinene, linalol, chrysanthenone in the flowers.

The amount and composition of the essential oil, according to the results obtained, and the existing data in specialized writings, vary depending on the parts of the plant (stems, leaves, flowers), phenophase, ecotype, temperature and light.

The anatomy elements looked at in this paper are very important for plant systematics and taxonomy, facilitating a correct diagnosis of the species.

The species *Artemisia absinthium* L. (Asteraceae) has distinctive traits, such as the cuticle showing a sinuous structure and the presence of simple and T-shaped non-glandular trichomes, as well as large and bicapitate glandular trichomes.

Knowledge of anatomy elements is also important due to the accumulation and production of secondary metabolites in the glandular trichomes.

The *A. absinthium* stems and leaves are covered with dense T-shaped glandular and non-glandular trichomes, on both sides, the most vigorous being those on the stem and petioles.

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RESEARCH ON THE STRUCTURE, ROLE AND ECOLOGICAL INDICATORS OF EPIGEIC ARTHROPOD SPECIES FOUND IN SOME SWEET CHERRY OCHARDS IN THE RĂDUCĂNENI AREA, IAȘI COUNTY, DEPENDING ON THE APPLIED CHEMICAL TREATMENTS

Romeo Ciprian BALINT[1], Monica HEREA[1], Ion MITREA[2], Renate SIPOS[1], Mihai TĂLMACIU[1]

[1]"Ion Ionescu de la Brad" Iaşi University of Life Sciences,
3 Mihail Sadoveanu Alley, Iași, Romania
[2]University of Craiova, 19 Libertății Street, Craiova, Romania

Corresponding author email: monica.herea@iuls.ro

Abstract

The research was conducted for a period of three years , in a cherry plantation in Răducăneni area, Iași County. The material was collected using Barber soil traps, inside the traps using a 2.5% NaCl solution to capture arthropod species. The collected material was cleaned, brought to the laboratory where the species were identified by harvest date, traps and variants. A number of 12 traps were used, installed in two rows at a distance of 12 m between rows and 6-8m between traps per row. In 2022, seven collections were made from May to October, and the most frequently collected species were: Harpalus distinguendus; Anisodactylus binotatus. In 2023, collections of arthropods from Barber traps were made and the most frequently collected species were: Anisodactylus signatus, Anisodactylus binotatus, Pseudophonus pubescens; Calathus fuscipes. In 2024, a total of 6 collections were made and the most frequently collected species were those belonging to the genera: Amara, Anisodactylus, Harpalus, Calathus, all belonging to the Carabidae family. In addition to insects, species of arachnids and millipedes were also collected.

Key words*: ecological indicators, sweet cherry ochards, arthropod species, chemical treatments.*

INTRODUCTION

Epigeal arthropods play an essential role in agricultural ecosystems by influencing the dynamics of pest populations and contributing to the maintenance of ecological balance. In fruit plantations, these communities are affected by a range of abiotic and biotic factors, including phytosanitary treatments applied to protect crops (Herea et al., 2010; 2011).

This study aims to investigate the structure, role, and ecological indicators of epigeal arthropods in cherry orchards in Iași County, depending on the chemical treatment regime used. It seeks to evaluate species diversity, abundance, and relevant ecological indicators to understand how these agricultural practices influence arthropod communities.

Research in this field is particularly important, given the need to develop sustainable crop protection strategies that minimize negative impacts on beneficial entomofauna (Perju et al., 2021). Through a comparative analysis of different types of treatments applied in cherry orchards, the study could contribute to the development of integrated pest management measures with a reduced impact on local biodiversity.

The impact of pests on fruit and vine crops results from the interaction between a specific host/parasite system and the local environmental and cultural conditions (Talmaciu et al., 2011). Due to the expansion of organic farming, changes have occurred in the spectrum of diseases and pests in agricultural crops, compared to conventional farming systems where chemical treatments are applied (Tezcan C. and Gülperçin N., 2024).

Since 1990, environmental protection, biodiversity conservation, and habitat preservation issues have gained a new dimension, resulting in the identification of new areas with productive potential in the horticultural, viticultural, and fruit-growing sectors. The need for biological and ecological knowledge in this field has become increasingly significant, especially since between 18,000 and 55,000 species disappear worldwide each year.

Beetles have various beneficial functions for humans; they are good indicators and can serve as one of the main tools in solving cadastral and integrated ecological monitoring problems. Based on their presence and frequency in ecosystems, various mathematical models of the dynamics of economically significant animals could be developed, and principles and pathways for predicting and managing different agricultural and forestry pests could be created.
Considering the position and role of beetles in different types of horticultural ecosystems, this research was necessary in two distinct types of ecosystems: fruit-growing and viticulture (Mitrea, 2011).

MATERIALS AND METHODS

The soil traps type Barber were used to collect the biological material. These consisted in the introduction into the soil of recipients in which a salt solution (2.5%) (Herea M., 2010).
The experience was organized in a sweet cherry orchard cultivated with Stela cultivar, which belongs to the Răducăneni stationary.
The location of the traps has been done on two rows at a distance of 12 m between the rows and 6 meters from the traps by three per row (Tălmaciu M., 2011).
The gathering of the entomological material was done between May of August at intervals of 10-20 days in each year of observations 2022, 2023, 2024.
At each harvest, the insects collected from each trap were put into gauze cloth, each sample separately, and the liquid in the trap was replaced or filled. The material was then labeled, on the label specifying: the date of collection; trap number and the variant.
In the laboratory, the material was cleaned from plant debris and then washed under the jet of water, selected by order or species.
As for the data interpretation, a number of ecological indices have been calculated as follows: abundance, constancy, dominance, and ecological significance.
Abundance (A) - Expresses the number of collected specimens.
Constancy (C) - represent the number of samples in which the species appears, based on the number of samples taken, according to the formulate:

$$C_A = \frac{N_P \cdot A \cdot 100}{N_P}$$

CA - constance of species
NpA - The number of traps in which species A appears;
Np - Total number of traps,
with 4 classes of constants:
C1 - Accidental species (1-2%);
C2 - Accessory species (25.1-50%);
C3 - Constant species (50.1-75%);
C4 - Euconstant species (75.1-100%).

Dominance (D) Is the total number of samples relative to the total number of individuals harvested according to the formula:

$$D_A = \frac{N_A \cdot 100}{N_t}$$

DA - constance of the A;
NA - total number the samples of A species;
Nt - total number of samples for all species,
with 5 dominance classes:
D1 - subrecedent (sub 1.1%);
D2 - recedent (1.1-2%);
D3 - subdominant (2.1-5%);
D4 - dominant (5.1-10%);
D5 - eudominant (above 10%).

The Significance Ecological Index (W) by formulate:

$$W_A = \frac{C_A \cdot D_A \cdot 100}{10000}$$

WA - ecological significance index of the A species;
CA - constance of the A species;
DA - dominance of the A species,
with 5 clases:
W1 - Less than 0.1% (Accidental species);
W2 - 0.1-1%;
W3 - 1.1-5% (Accompanying species);
W4 - 5.1-10%;
W5 - aove 10% (Edifying species).
The Ecological Significance Index (W) is used to deepen the interrelationships between constancy and dominance, emphasizing even more clearly the structure of some species, reflecting its importance in the analyzed community.

RESULTS AND DISCUSSIONS

To achieve the proposed objectives and following the activities carried out, seven harvests were conducted at the Răducăneni station using six soil traps, with a total of 84

such traps being used. These traps collected 428 specimens belonging to the epigeic fauna in the cherry plantation in 2022.

To achieve this goal, the sinecological analysis of fauna on sweet cherry orchards was performed to assess the ratio of each species in the analyzed biocenosis.

The material obtained from the observations was mathematically processed to obtain a series of ecological indicators: abundance (A), dominance (D), constancy (C) and ecological significance index (W).

Table 1.Species composition of arthropods collected in soil traps in 2022 in the cherry orchard

No.	Name of species	Harvested							Number of traps	Total samples
		I	II	III	IV	V	VI	VII		
1.	*Phalangium opilo*	0	19	24	9	20	13	14	32	99
2.	*Anisodactylus binotatus*	30	21	9	15	2	0	2	27	79
3.	*Harpalus distinguendus*	29	18	4	8	4	1	1	30	65
4.	*Dermestes laniarius*	4	5	2	1	6	1	0	14	17
5.	*Harpalus tardus*	6	8	2	0	1	0	0	7	17
6.	*Licinus cassideus*	0	0	0	0	0	6	7	10	13
7.	*Amara aenea*	4	3	2	0	2	0	0	8	11
8.	*Amara apricaria*	0	9	0	0	1	1	0	5	11
9.	*Carabus coriaceus*	0	3	2	2	0	0	3	7	10
10.	*Anisodactylus signatus*	0	0	0	0	9	1	0	5	10
11.	*Pseudophonus pubescens*	0	0	0	0	2	4	3	7	9
12.	*Cartodere elongata*	0	0	0	0	0	1	8	4	9
13.	*Ophonus azureus*	4	2	0	0	1	1	0	6	8
14.	*Chromatoiulus unilineatus*	3	2	0	0	3	0	0	5	8
15.	*Armadilidium vulgare*	3	0	0	0	0	1	3	7	7
16.	*Amara familiaris*	4	0	0	0	1	0	0	3	5
17.	*Forficula auricularia*	0	1	3	0	0	0	0	3	4
18.	*Ophonus puncticollis*	0	0	0	4	0	0	0	2	4
19.	*Pseudophonus griseus*	2	1	0	0	0	0	0	3	3
20.	*Brachynus crepitans*	2	0	0	0	0	0	0	2	2
21.	*Harpalus calceatus*	1	1	0	0	0	0	0	2	2
22.	*Amara similata*	0	0	2	0	0	1	0	2	3
23.	*Amara crenata*	0	0	0	1	0	0	1	2	2
24.	*Blaps mortisaga*	0	0	0	1	1	0	0	2	2
25.	*Leistus ferrugineus*	0	0	0	2	0	0	0	1	2
26.	*Metabletus truncatellus*	0	0	0	1	1	0	0	2	2
27.	*Ophonus sabulicolla*	0	0	0	1	0	1	0	2	2
28.	*Harpalus rufus*	0	0	0	0	0	1	1	1	2
29.	*Otiorrhynchus pinastri*	0	0	0	0	0	2	0	2	2
30.	*Quedus cinctus*	0	0	0	0	0	0	2	1	2
31.	*Amara eurynota*	1	0	0	0	0	0	0	1	1
32.	*Colodera aethiops*	1	0	0	0	0	0	0	1	1
33.	*Tachyporus abdominalis*	1	0	0	0	0	0	0	1	1
34.	*Pentodon idiota*	0	1	0	0	0	0	0	1	1
35.	*Carabus violaceus*	0	0	1	0	0	0	0	1	1
36.	*Lathrobium quadratum*	0	0	1	0	0	0	0	1	1
37.	*Lathrobium multipunctatum*	0	0	0	0	0	1	0	1	1
38.	*Pterostichus cupreus*	0	0	1	0	0	0	0	1	1
39.	*Sericus bruneus*	0	0	1	0	0	0	0	1	1
40.	*Calathus fuscipes*	0	0	0	1	0	0	0	1	1
41.	*Cypticus quisquilius*	0	0	0	0	1	0	0	1	1
42.	*Ontophagus ovatus*	0	0	0	0	1	0	0	1	1
43.	*Opatrum sabulosum*	0	0	0	0	1	0	0	1	1
44.	*Panagaeus crux-major*	0	0	0	0	1	0	0	1	1
45.	*Staphilinus caesareus*	0	0	0	0	1	0	0	1	1
46.	*Harpalus autumnalis*	0	0	0	0	0	1	0	1	1
47.	*Calathus melanocephalus*	0	0	0	0	0	0	1	1	1
48.	*Pterostichus nigrita*	0	0	0	0	0	0	1	1	1
49.	*Triplax lepida*	0	0	0	0	0	0	1	1	1
Total 49 species		95	94	54	46	59	37	48		427

The data in Table 1 reflect the seasonal variations of the captured arthropod fauna, highlighting maximum activity during the spring months when the highest number of captures were recorded.

In 2022, in the cherry orchard in Răducăneni, 427 arthropod specimens were collected, belonging to 49 species, using soil traps. The most frequent species were *Phalangium opilo* (99 specimens), *Anisodactylus binotatus* (79

specimens), and *Harpalus distinguendus* (65 specimens). The highest catches were recorded in the first two samplings (95 and 94 specimens), while the lowest occurred in the sixth sampling (37 specimens). This high diversity suggests a stable and balanced habitat, influenced by climatic factors and the specific nature of the cherry orchard agroecosystem. The obtained data can contribute to future strategies for sustainable ecosystem management, maintaining biodiversity and promoting beneficial species.

Table 2. Species composition of arthropods collected in soil traps in 2022 in the cherry orchard

No.	Name of species	Number of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Phalangium opilo*	32	99	38,1	C2	23,1	D5	8,801	W5
2.	*Anisodactylus binotatus*	27	79	32,1	C2	18,5	D5	5,938	W4
3.	*Harpalus distinguendus*	30	65	35,7	C2	15,2	D5	5,426	W4
4.	*Dermestes laniarius*	14	17	1,7	C1	4,0	D3	0,008	W1
5.	*Harpalus tardus*	7	17	8,3	C1	4,0	D3	0,332	W2
6.	*Licinus cassideus*	10	13	11,9	C1	30,4	D5	3,618	W3
7.	*Amara aenea*	8	11	9,5	C1	2,6	D3	0,247	W2
8.	*Amara apricaria*	5	11	6,0	C1	2,6	D3	0,156	W2
9.	*Carabus coriaceus*	7	10	8,3	C1	23,3	D5	1,934	W3
10.	*Anisodactylus signatus*	5	10	6,0	C1	23,3	D5	1,398	W3
11.	*Pseudophonus pubescens*	7	9	8,3	C1	2,1	D3	0,174	W3
12.	*Cartodere elongata*	4	9	4,7	C1	2,1	D3	0,099	W1
13.	*Ophonus azureus*	6	8	7,1	C1	1,9	D2	0,134	W2
14.	*Chromatoiulus unilineatus*	5	8	6,0	C1	1,9	D2	0,144	W2
15.	*Armadilidium vulgare*	7	7	8,3	C1	1,6	D2	0,132	W2
16.	*Amara familiaris*	3	5	3,5	C1	1,2	D2	0,042	W1
17.	*Forficula auricularia*	3	4	3,5	C1	0,9	D1	0,031	W1
18.	*Ophonus puncticollis*	2	4	2,4	C1	0,9	D1	0,021	W1
19.	*Pseudophonus griseus*	3	3	3,5	C1	0,7	D1	0,024	W1
20.	*Brachynus crepitans*	2	2	2,4	C1	0,5	D1	0,012	W1
21.	*Harpalus calceatus*	2	2	2,4	C1	0,5	D1	0,012	W1
22.	*Amara similata*	3	2	3,5	C1	0,5	D1	0,017	W1
23.	*Amara crenata*	2	2	2,4	C1	0,5	D1	0,012	W1
24.	*Blaps mortisaga*	2	2	2,4	C1	0,5	D1	0,012	W1
25.	*Leistus ferrugineus*	1	2	1,2	C1	0,5	D1	0,006	W1
26.	*Metabletus truncatellus*	2	2	2,4	C1	0,5	D1	0,012	W1
27.	*Ophonus sabulicolla*	2	2	2,4	C1	0,5	D1	0,012	W1
28.	*Harpalus rufus*	1	2	1,2	C1	0,5	D1	0,006	W1
29.	*Otiorrhynchus pinastri*	2	2	2,4	C1	0,5	D1	0,012	W1
30.	*Quedus cinctus*	1	2	1,2	C1	0,5	D1	0,006	W1
31.	*Amara eurynota*	1	1	1,2	C1	0,2	D1	0,002	W1
32.	*Colodera aethiops*	1	1	1,2	C1	0,2	D1	0,002	W1
33.	*Tachyporus abdominalis*	1	1	1,2	C1	0,2	D1	0,002	W1
34.	*Pentodon idiota*	1	1	1,2	C1	0,2	D1	0,002	W1
35.	*Carabus violaceus*	1	1	1,2	C1	0,2	D1	0,002	W1
36.	*Lathrobium quadratum*	1	1	1,2	C1	0,2	D1	0,002	W1
37.	*Lathrobium multipunctatum*	1	1	1,2	C1	0,2	D1	0,002	W1
38.	*Pterostichus cupreus*	1	1	1,2	C1	0,2	D1	0,002	W1
39.	*Sericus bruneus*	1	1	1,2	C1	0,2	D1	0,002	W1
40.	*Calathus fuscipes*	1	1	1,2	C1	0,2	D1	0,002	W1
41.	*Cypticus quisquilius*	1	1	1,2	C1	0,2	D1	0,002	W1
42.	*Ontophagus ovatus*	1	1	1,2	C1	0,2	D1	0,002	W1
43.	*Opatrum sabulosum*	1	1	1,2	C1	0,2	D1	0,003	W1
44.	*Panagaeus crux-major*	1	1	1,2	C1	0,2	D1	0,002	W1
45.	*Staphilinus caesareus*	1	1	1,2	C1	0,2	D1	0,002	W1
46.	*Harpalus autumnalis*	1	1	1,2	C1	0,2	D1	0,002	W1
47.	*Calathus melanocephalus*	1	1	1,2	C1	0,2	D1	0,002	W1
48.	*Pterostichus nigrita*	1	1	1,2	C1	0,2	D1	0,002	W1
49.	*Triplax lepida*	1	1	1,2	C1	0,2	D1	0,002	W1
Total 49 species		84	428						

From an ecological perspective, the data in Table 2 reflect the diversity and structure of the species community observed in a specific area, likely within a faunistic-ecological study. These species are evaluated based on four main indicators: abundance (A), constancy (C), dominance (D), and ecological significance index (W).

Abundance represents the total number of specimens for each species. The most abundant

species are *Phalangium opilio*, *Anisodactylus binotatus*, and *Harpalus distinguendus*, suggesting a clear dominance of these species in the studied ecosystem. These species can be considered indicators of a stable environment favorable to their development.

Constancy reflects the frequency of occurrence of each species in a significant number of traps throughout the study. Species with higher constancy (such as *Phalangium opilio*, *Anisodactylus binotatus*, and *Harpalus distinguendus*) are considered the most constant, indicating that these species are more resilient and better adapted to local ecological conditions.

Dominance indicates the proportion of the species within an ecosystem and is a measure of its ecological influence. Species with high dominance, such as *Phalangium opilio* (23.1%), *Anisodactylus binotatus* (18.5%), and *Harpalus distinguendus* (15.2%), play a significant role in the ecological structure of the studied area. These species, with a large proportion of the total number of specimens, significantly influence resource dynamics, interspecific relationships, and predation.

The ecological significance index (W) reflects the global ecological importance of each species and is calculated based on abundance, constancy, and dominance. Species with high values, such as *Phalangium opilio* (8.801), *Anisodactylus binotatus* (5.938), and *Harpalus distinguendus* (5.426), are the most ecologically influential. These species have a considerable impact on the ecosystem's balance, affecting available resources and interactions with other species. On the other hand, species such as *Dermestes laniarius* (W=0.008) and *Harpalus tardus* (W=0.332) have much lower ecological values, suggesting that they have a reduced ecological impact and lower adaptability compared to others.

The arthropod community in the cherry plantation is dominated by a few key species, particularly *Phalangium opilio*, which is both highly abundant (W5) and dominant (D5). This species contributes significantly to the community structure, accounting for 23.1% of total abundance. Other important species include *Anisodactylus binotatus* and *Harpalus distinguendus*, both classified as C2 (Accessory species) and D5 (Eudominant), with moderate abundance (W4). Most species are C1 (Accidental species) and D1 (Subrecedent), contributing less than 1.1% of the total abundance. These species are sporadically present and have minimal ecological impact. Overall, a few dominant species shape the ecosystem, while many others appear occasionally with little contribution to total abundance.

Following the centralization of the data collected in 2023 from the cherry plantation in the Răducăneni station, the epigeal entomofauna was represented by 49 species, with 499 specimens from the 132 traps used between May and September.

Table 3. Species composition of arthropods collected in soil traps in 2023 in the cherry orchard

No.	Name of species											Number of traps	Total samples
		II	III	IV	V	VI	VII	VIII	IX	X	XI		
1.	*Phalangium opilio*	39	33	15	47	11	4	13	9	3	4	68	175
2.	*Anisodactylus binotatus*	36	0	8	10	20	7	2	0	0	0	31	81
3.	*Harpalus atratus*	0	24	0	0	0	0	0	0	0	0	7	24
4.	*Dermestes laniarius*	6	4	3	3	1	0	0	0	2	0	16	19
5.	*Harpalus distinguendus*	6	2	6	3	2	0	0	0	0	0	13	19
6.	*Harpalus tardus*	0	11	2	2	0	3	0	0	0	1	12	19
7.	*Amara aenea*	8	8	0	0	0	0	0	0	0	0	8	16
8.	*Pseudophonus pubescens*	1	0	0	0	0	0	4	5	0	4	9	14
9.	*Ophonus azureus*	1	1	0	1	2	4	1	2	0	1	12	13
10.	*Amara similata*	8	1	0	1	1	0	0	0	0	0	6	11
11.	*Brachynus crepitans*	3	0	0	0	2	3	2	0	0	0	8	10
12.	*Licinus cassideus*	1	1	0	0	1	2	1	0	0	2	8	8
13.	*Amara familiaris*	6	0	0	0	2	0	0	0	0	0	3	8
14.	*Amara apricaria*	5	0	1	1	0	0	0	0	0	0	5	7
15.	*Amara crenata*	4	0	0	0	1	0	0	0	0	1	4	6
16.	*Chromoloulus unilineatus*	5	0	1	0	0	0	0	0	0	0	4	6
17.	*Carabus coriaceus*	0	4	0	1	0	0	0	0	0	1	6	6
18.	*dermaptere*	0	1	0	3	0	1	0	0	0	0	6	6
19.	*Anisodactylus signatus*	0	0	0	4	0	0	0	0	2	0	2	6
20.	*Amara eurynota*	4	0	0	0	0	0	0	0	0	0	2	4
21.	*Brachynus explodens*	0	0	0	0	0	1	1	1	0	0	3	3

22.	*Opatrum sabulosum*	0	2	1	0	0	0	0	0	0	0	2	3
23.	*Calathus fuscipes*	0	1	0	0	1	0	0	0	0	0	2	2
24.	*Harpalus calceatus*	0	1	0	0	0	0	0	0	1	0	2	2
25.	*Blaps mortisaga*	0	0	0	1	0	0	0	0	1	0	2	2
26.	*Leistus ferrugineus*	0	0	0	1	1	0	0	0	0	0	2	2
27.	*Armadilidium vulgare*	0	0	0	0	1	0	0	0	0	1	2	2
28.	*Pterostichus cupreus*	0	0	0	0	2	0	0	0	0	0	1	2
29.	*Bledius talpa*	1	0	0	0	0	0	0	0	0	0	1	1
30.	*Forficula auricularia*	1	0	0	0	0	0	0	0	0	0	5	1
31.	*Harpalus hirtipes*	6	0	0	0	0	0	0	0	0	0	6	1
32.	*Harpalus neglectus*	1	0	0	0	0	0	0	0	0	0	1	1
33.	*Staphylinius pedator*	1	0	0	0	0	0	0	0	0	0	1	1
34.	*Pentodon idiota*	0	1	0	0	0	0	0	0	0	0	1	1
35.	*Metabletus truncatellus*	0	0	1	0	0	0	0	0	0	0	1	1
36.	*Ophonus sabulicola*	0	0	1	0	0	0	0	0	0	0	1	1
37.	*Airaphilus corsicus*	0	0	0	1	0	0	0	0	0	0	1	1
38.	*Otiorhynchus pinastri*	0	0	0	0	1	0	0	0	0	0	1	1
39.	*Staphylinus caesareus*	0	0	0	0	1	0	0	0	0	0	1	1
40.	*Crypticus quisquilius*	0	0	0	0	0	0	0	1	0	0	1	1
41.	*Staphylinus olens*	0	0	0	0	0	0	0	1	0	0	1	1
Total 41 species		143	96	39	79	50	25	24	19	9	15	132	499

The data in Table 3 reflect the structure of the arthropod fauna collected in soil traps from the cherry orchard in 2023. The most abundant species are *Phalangium opilio* (175 specimens), followed by *Anisodactylus binotatus* (81 specimens), and (16 specimens). These species are dominant and present throughout several samplings, suggesting that the habitat is favorable. On the other hand, many species have a small number of specimens, indicating a rarer presence in the ecosystem. A total of 41 species were identified, with a diversity ranging from common and abundant species to some that are rarer and have a very limited distribution.

Table 4. Species composition of arthropods collected in soil traps in 2023 in the cherry orchard

No.	Name of species	Number of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Phalangium opilio*	68	175	51,5	C3	35,1	D5	18,076	W5
2.	*Anisodactylus binotatus*	31	81	23,5	C1	16,2	D5	3,807	W3
3.	*Harpalus atratus*	7	24	5,3	C1	4,8	D3	0,254	W2
4.	*Dermestes laniarius*	16	19	12,1	C1	3,8	D3	0,459	W2
5.	*Harpalus distinguendus*	13	19	9,8	C1	3,8	D3	0,372	W2
6.	*Harpalus tardus*	12	19	9,1	C1	3,8	D3	0,345	W2
7.	*Amara aenea*	8	16	6,1	C1	3,2	D3	0,195	W2
8.	*Pseudophonus pubescens*	9	14	6,8	C1	2,8	D3	0,190	W2
9.	*Ophonus azureus*	12	13	9,1	C1	2,6	D3	0,237	W2
10.	*Amara similata*	6	11	4,5	C1	2,2	D3	0,099	W1
11.	*Brachynus crepitans*	8	10	6,1	C1	2,0	D2	0,122	W2
12.	*Amara familiaris*	3	8	2,3	C1	1,6	D2	0,036	W1
13.	*Licinus cassideus*	8	8	6,1	C1	1,6	D2	0,096	W1
14.	*Amara apricaria*	5	7	3,7	C1	1,4	D2	0,042	W1
15.	*Amara crenata*	4	6	3,0	C1	1,2	D2	0,036	W1
16.	*Anisodactylus signatus*	2	6	1,5	C1	1,2	D2	0,018	W1
17.	*Carabus coriaceus*	6	6	4,5	C1	1,2	D2	0,054	W1
18.	*Chromoloulus unilineatus*	4	6	3,0	C1	1,2	D2	0,036	W1
19.	*dermaptere*	6	6	4,5	C1	1,2	D2	0,054	W1
20.	*Amara eurynota*	2	4	1,5	C1	0,8	D1	0,012	W1
21.	*Brachynus explodens*	3	3	2,3	C1	0,6	D1	0,014	W1
22.	*Opatrum sabulosum*	2	3	1,5	C1	0,6	D1	0,009	W1
23.	*Armadilidium vulgare*	2	2	1,5	C1	0,4	D1	0,006	W1
24.	*Blaps mortisaga*	2	2	1,5	C1	0,4	D1	0,006	W1
25.	*Calathus fuscipes*	2	2	1,5	C1	0,4	D1	0,006	W1
26.	*Harpalus calceatus*	2	2	1,5	C1	0,4	D1	0,006	W1
27.	*Leistus ferrugineus*	2	2	1,5	C1	0,4	D1	0,006	W1
28.	*Pterostichus cupreus*	1	2	0,8	C1	0,4	D1	0,006	W1
29.	*Airaphilus corsicus*	1	1	0,8	C1	0,2	D1	0,002	W1
30.	*Bledius talpa*	1	1	0,8	C1	0,2	D1	0,002	W1
31.	*Crypticus quisquilius*	1	1	0,8	C1	0,2	D1	0,002	W1
32.	*Forficula auricularia*	5	1	3,7	C1	0,2	D1	0,007	W1
33.	*Harpalus hirtipes*	6	1	4,5	C1	0,2	D1	0,009	W1
34.	*Harpalus neglectus*	1	1	0,8	C1	0,2	D1	0,002	W1
35.	*Metabletus truncatellus*	1	1	0,8	C1	0,2	D1	0,002	W1

36.	*Ophonus sabulicola*	1	1	0,8	C1	0,2	D1	0,002	W1
37.	*Otiorhynchus pinastri*	1	1	0,8	C1	0,2	D1	0,002	W1
38.	*Pentodon idiota*	1	1	0,8	C1	0,2	D1	0,002	W1
39.	*Staphylinius pedator*	1	1	0,8	C1	0,2	D1	0,002	W1
40.	*Staphylinus caesareus*	1	1	0,8	C1	0,4	D1	0,006	W1
41.	*Staphylinus olens*	1	1	0,8	C1	0,2	D1	0,002	W1
Total 41 species		132	499						

In the cherry orchard, in 2023 (Table 4) the structure of the arthropod fauna collected in soil traps was dominated by only a few species, but with a high diversity of species. *Phalangium opilio* was the most abundant species, with 175 individuals, followed by *Anisodactylus binotatus* with 81 individuals. These species were also the most constant (C3 and C1) and had the highest dominance class (D5), significantly impacting the ecological structure of the orchard ecosystem.

âMany species, such as *Amara aenea*, *Dermestes laniarius*, and *Amara similata*, had low abundance, indicating high ecological diversity but with dominance concentrated around a few species.

Most species were rare, with only a few specimens, suggesting that they do not significantly influence the community dynamics.

In the dataset, *Phalangium opilio* is the dominant and most abundant species (C3, D5, W5), significantly shaping the community. Most species are C1 (Accidental), with low constancy and abundance, indicating infrequent presence and minimal ecological impact. Species like *Anisodactylus binotatus* and *Harpalus atratus* are D5 (Eudominant) but still have lower overall presence, while others like *Amara similata* and *Brachynus explodens* are D1 (Subrecedent), contributing little to the community. Overall, the community is mainly composed of sporadic, low-impact species, with a few dominant ones.

In the third year of observation regarding the epigeal fauna in the cherry orchards, a total of 6 samplings were carried out using 72 traps.

Table 5. Species composition of arthropods collected in soil traps in 2024 in the cherry orchard

No.	**Name of species**	**Harvested**						**No. of traps**	**Total samples**
		I	**II**	**III**	**IV**	**V**	**VI**		
1.	*Phalangium opilio*	23	35	20	62	31	9	47	180
2.	*Forficula auricularia*	1	0	19	0	1	0	7	21
3.	*Pseudophonus pubescens*	0	0	2	7	6	5	14	20
4.	*Harpalus distinguendus*	8	7	1	0	0	0	9	16
5.	*Anisodactyls binotatus*	0	4	0	7	3	0	6	14
6.	*Harpalus tardus*	5	5	0	0	0	0	8	10
7.	*Amara familiaris*	5	1	0	0	1	0	6	7
8.	*Amara aenea*	1	0	1	2	2	0	6	6
9.	*Anisodactyls signatus*	4	0	0	0	0	0	1	4
10.	*Amara eurynota*	2	0	0	0	0	0	2	2
11.	*Dermestes laniarius*	2	0	0	0	0	0	2	2
12.	*Chromoloulus unilineatus*	2	0	0	1	0	0	1	2
13.	*Otiorrhyncus scaber*	2	0	0	0	0	0	1	2
14.	*Pseudophonus pubescens*	2	0	0	0	0	0	2	2
15.	*Pterosticus vulgaris*	1	0	0	0	0	0	1	1
16.	*Rhynchites pausillus*	1	0	0	0	0	0	1	1
17.	*Calathus fuscipes*	0	1	0	0	0	0	1	1
18.	*Harpalus rufus*	0	1	0	0	0	0	1	1
19.	*Apion rufirostre*	0	0	1	0	0	0	1	1
20.	*Harpalus calceatus*	0	0	1	0	0	0	1	1
21.	*Dorcus paralelipipedes*	0	0	0	1	0	0	1	1
22.	*Ontophagus orlatus*	0	0	0	1	0	0	1	1
23.	*Ophonus diffinis*	0	0	0	1	0	0	1	1
24.	*Carabus coriaceus*	0	0	0	1	0	0	1	1
25.	*Ophonus puncticalis*	0	0	0	1	0	0	1	1
26.	*Poecilus cupreus*	0	0	0	1	0	0	1	1
Total 26 species		59	54	45	81	47	15		72

The Table 5 shows species distribution across different sampling periods. *Arahnids* dominates with high numbers, especially in periods I and II. *Forficula auricularia* and *Pseudophonus pubescens* appear sporadically, with notable presence in the III, IV, and V harvest. Species

like *Harpalus distinguendus* and *Anisodactyls binotatus* show peaks in specific periods. *Amara familiaris***,** *Amara aenea*, and *Anisodactyls signatus* are less frequent, appearing in select periods. Other species, such as *Dermestes laniarius* and *Otiorrhyncus scaber*, are rare, found only in a few periods. Species distribution is uneven, with some dominating and others appearing irregularly.

Table 6. Species composition of arthropods collected in soil traps in 2024 in the cherry orchard

No.	Name of species	No. of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Phalangium opilio*	47	180	65,2	C3	59,8	D5	38,989	W5
2.	*Forficulla auricularia*	7	21	9,7	C1	7,0	D4	0,679	W2
3.	*Pseudophonus pubescens*	14	20	19,4	C1	6,6	D4	1,280	W3
4.	*Harpalus distinguendus*	9	16	12,5	C1	5,3	D4	0,662	W2
5.	*Anisodactyls binotatus*	6	14	8,3	C1	4,6	D3	0,381	W2
6.	*Harpalus tardus*	8	10	11,2	C1	3,3	D3	0,369	W2
7.	*Amara familiaris*	6	7	8,3	C1	2,3	D3	0,0190	W2
8.	*Amara aenea*	6	6	8,3	C1	2,0	D2	0,166	W2
9.	*Anisodactyls signatus*	1	4	1,4	C1	1,3	D2	0,018	W1
10.	*Chromoloulus unilineatus*	2	3	2,8	C1	1,0	D1	0,028	W1
11.	*Amara eurynota*	2	2	2,8	C1	0,7	D1	0,019	W1
12.	*Dermestes laniarius*	2	2	2,8	C1	0,7	D1	0,019	W1
13.	*Otiorrhyncus scaber*	1	2	1,4	C1	0,7	D1	0,009	W1
14.	*Pseudophonus pubescens*	2	2	2,8	C1	0,7	D1	0,019	W1
15.	*Pterosticus vulgaris*	1	1	1,4	C1	0,3	D1	0,004	W1
16.	*Rhynchites pausillus*	1	1	1,4	C1	0,3	D1	0,004	W1
17.	*Calathus fuscipes*	1	1	1,4	C1	0,3	D1	0,004	W1
18.	*Harpalus rufus*	1	1	1,4	C1	0,3	D1	0,004	W1
19.	*Apion rufirostre*	1	1	1,4	C1	0,3	D1	0,004	W1
20.	*Harpalus calceatus*	1	1	1,4	C1	0,3	D1	0,004	W1
21.	*Dorcus paralelipipedes*	1	1	1,4	C1	0,3	D1	0,004	W1
22.	*Ontophagus orlatus*	1	1	1,4	C1	0,3	D1	0,004	W1
23.	*Ophonus diffinis*	1	1	1,4	C1	0,3	D1	0,004	W1
24.	*Carabus coriaceus*	1	1	1,4	C1	0,3	D1	0,004	W1
25.	*Ophonus puncticalis*	1	1	1,4	C1	0,3	D1	0,004	W1
26.	*Poecilus cupreus*	1	1	1,4	C1	0,3	D1	0,004	W1
Total 28 species		72	301						

In the provided table no. 6, the *Phalangium opilio* stands out as the most abundant species with high constancy (C3) and dominance (D5), having a significant ecological impact (W5). It is a key species, dominating the community both in terms of numbers and relative frequency.

Other species, such as *Forficula auricularia, Pseudophonus pubescens,* and *Harpalus distinguendus*, show moderate abundance and constancy (C1), with dominance ranging from D4 to D3, contributing notably but less than *Phalangium opilio*. These species fall into the W2 to W3 categories, indicating their presence as important but not overwhelming contributors to the ecosystem.

Most other species, including *Amara familiaris, Anisodactylus binotatus*, and *Amara aenea*, show low constancy and dominance (C1, D2, D3), with very low ecological impact (W1). These species contribute minimally to the community and have sporadic occurrences.

Overall, the community is dominated by a few species with high abundance and ecological relevance, while many others are sporadic and contribute minimally to the structure of the ecosystem.

The graph shows the evolution of the number of individuals for different species between 2022 and 2024. *Phalangium opilio* is the most abundant species in all three years, with a significant increase in 2023 and maintaining a high number in 2024. Other species, such as *Anisodactylus binotatus* and *Harpalus distinguendus*, show fluctuations, with some experiencing significant declines in 2024. New species also appear, such as *Forficula auricularia* and *Pseudophonus pubescens*, which were previously absent or present in very small numbers. This variation may indicate environmental changes or ecological factors influencing these populations.

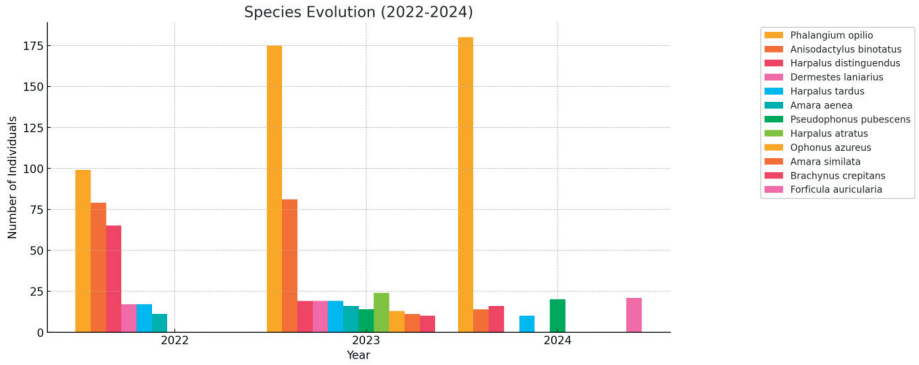


Figure 1. Comparative representation of the number of individuals for the most abundant species in the period 2022-2024

CONCLUSIONS

It was found that *Phalangium opilio* is the most abundant species, with high constancy (C3) and high dominance (D5), having a significant ecological impact (W5), being essential for the community structure and significantly influencing the orchard ecosystem.

Following the analysis of ecological indices, it was confirmed that species such as *Forficula auricularia*, *Pseudophonus pubescens*, and *Harpalus distinguendus* have moderate abundance and constancy (C1), with dominance ranging from D4 to D3, having a moderate ecological impact (W2-W3). These species play an important role but are not dominant in shaping the ecosystem.

Many species, such as *Amara familiaris*, *Anisodactylus binotatus*, and *Amara aenea*, have low constancy and dominance (C1, D2, D3), with very low ecological impact (W1), contributing minimally to community dynamics and being rarely present.

The arthropod species collected and identified in the cherry plantation are characterized by high species diversity, but with a small number of dominant species that form the main structure of the ecosystem, while most species have a low impact on it. It can be observed that the dominance of species such as *Phalangium opilio* suggests a stable and favorable habitat for their development, while the rest of the species play a smaller role in maintaining the ecological balance of the plantation.

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STUDY ON THE STRUCTURE, DYNAMICS, ABUNDANCE AND OTHER ECOLOGICAL INDICATORS OF ARTHROPOD SPECIES IN PEA CROPS IN NORTH-EAST ROMANIA

Răducu Ionuţ BALINT[1], Monica HEREA[1], Nela TĂLMACIU[1], Ionela MOCANU[1], Ion MITREA[2], Mihai TĂLMACIU[1]

[1]"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania
[2]University of Craiova, 19 Libertăţii Street, Craiova, Romania

Corresponding author email: monica.herea@iuls.ro

Abstract

The research was carried out during two years, 2023 and 2024, in a pea crop located in the north-east of Romania, in the Răducăneni locality of Iaşi county. In the study, the pitfall traps, filled with a 2.5% NaCl solution. We are used 12 traps, arranged in two rows of 6 traps for each, to carry out the research. The traps were placed at distances of 8-10 meters between them, and a distance of 12 meters was maintained between rows. The material collected in the traps was appropriately labeled, each label indicating the trap number and the date of harvest. In 2023, the collection of material from traps was carried out on 10 harvested, but in 2024, a number of 6 harvests of the material from the traps were carried out. The species most frequently collected and which also had the most specimens collected were Opatrum sabulosum, Coccinella 7 punctata, Dermestes laniarius, orthopters species, Arachnids etc.

Key words*: ecological indicators, pea crops, arthropod species.*

INTRODUCTION

The study of arthropods in agricultural ecosystems is an area of interest in applied ecology, with significant implications for understanding the dynamics and stability of these ecosystems.

The study of epigeic fauna in agroecosystems is an essential aspect of ecological and agricultural research, directly impacting the dynamics of pest and predator populations, as well as soil health (Malachi and Mustea, 1993; Sabluk et al., 2021).

In this context, pea crops (*Pisum sativum* L.) provide a diverse habitat for numerous arthropod groups, including beetles and spiders, which play an important role in regulating harmful populations.

The results obtained can provide relevant information for the sustainable management of crops and biodiversity conservation, with practical implications for plant protection and reducing pesticide use through the promotion of natural biological control.

In this context, pea crops (*Pisum sativum* L.) offer a diverse habitat for numerous arthropod groups, including beetles and spiders, which play an important role in regulating harmful populations (Maneva et al., 2023).

The technology for cultivating peas involves several essential stages, from soil preparation and sowing to harvesting and phytosanitary protection. Peas prefer well-drained soils with a neutral to slightly acidic pH, and proper soil preparation is necessary to ensure good germination and plant development. Sowing is done in early spring at depths of 4-6 cm, with an optimal density that varies depending on the variety and the intended use of the crop.

Peas are a leguminous crop of great economic and agricultural importance, due to their nutritional value and their role in improving soil fertility (Rusch et al., 2016).

Due to their ability to fix nitrogen biologically through symbiotic bacteria (*Rhizobium*), peas contribute to reducing the need for nitrogen fertilizers and increasing soil productivity. They are used both for human consumption, in the form of dried or fresh beans, and as animal feed, being high in protein, fiber, and vitamins.

MATERIALS AND METHODS

This study aims to identify the predominant species, assess their diversity and roles within the agricultural ecosystem, and determine the factors that influence these ecological traits (Popov et al., 2005).

Barber traps are an effective and widely used method for collecting and monitoring epigeic fauna, using bowls buried at ground level and filled to 70% with a 2.5% NaCl solution. This method provided valuable data on the composition, abundance, distribution, dominance, and ecological significance of species in pea crops (Tălmaciu et al., 2019).

By using this method, the present study aims to analyze the diversity and structure of epigeic arthropod communities in pea crops, highlighting the ecological factors that influence these populations and contributing to a better understanding of the ecological interactions within the crop.

Pea crops (*Pisum sativum*) in northeastern Romania provide a suitable habitat for a wide range of arthropod species, which can affect both agricultural production and the ecological structure of these crops. Arthropods play an essential role in ecological processes such as pollination, natural pest control, and nutrient recycling, but they can also become limiting factors if their population increases uncontrollably.

In the stationary site represented by a pea crop cultivated in the Raducaneni commune during the two years of observation (2023-2024), the primary focus was on evaluating and centralizing the faunal structure to identify the insect groups that pose problems in this crop, as well as the beneficial fauna within this ecosystem.

This study represents an important contribution to understanding the complexity of the relationships between arthropods and pea crops, with the potential to support the development of more environmentally friendly agricultural practices.

The results obtained can provide relevant information for the sustainable management of crops and biodiversity conservation, with practical implications for plant protection and reducing pesticide use by promoting natural biological control.

RESULTS AND DISCUSSIONS

An essential role in the ecological balance of agricultural crops is played by the entomofauna in these agroecosystems, considering both beneficial and harmful species. In pea crops (*Pisum sativum*), the presence of insects can influence productivity through direct effects and interactions with other environmental factors.

Entomofauna in pea crops plays a crucial role in the agricultural ecology of this ecosystem, including both beneficial and harmful species. Using the Barber method for insect collection, this study analyzes their diversity and abundance, highlighting both beneficial and harmful species that influence the pea crop.

Table 1. The situation regarding the number of collections in which each species was collected during the period May-September, in 2023, and the type of food

No.	Name of species	Harvest										No. of traps	Total samples	Type of food***
		I	II	III	IV	V	VI	VI	VIII	IX	X			
1.	*Dermestes laniarius*	55	104	45	17	2	8	6	34	2	4	55	277	P
2.	*Messor structor*	10	20	9	0	52	144	20	21	5	10	33	274	P
3.	*Coccinella septempunctata*	128	74	29	33	0	0	0	0	0	0	34	201	P
4.	*Phalangium opilio*	46	15	36	17	3	10	7	15	2	2	50	151	P
5.	*Pseudophonus pubescens*	4	0	0	0	6	3	3	5	1	122	26	136	P
6.	*Dermestes lardarius*	22	20	18	0	0	0	0	0	0	0	15	60	P
7.	*Opatrum sabulosum*	21	27	11	0	0	0	0	0	0	1	25	59	F
8.	*Pyrrhocoris apterus*	14	3	0	0	0	0	0	0	6	6	14	29	F
9.	*Lixus cardui*	0	0	1	0	0	0	4	11	0	10	8	26	F
10.	*Hipodamia variegata*	4	15	0	1	1	0	0	0	0	0	14	22	P
11.	*Anthicus floralis*	0	0	20	0	0	0	0	0	0	0	5	20	P
12.	*Harpalus distinguendus*	14	1	0	3	2	0	0	0	0	0	9	20	D
13.	*Sitona lineatus*	20	0	0	0	0	0	0	0	0	0	2	20	I
14.	*Polydrusus amoenus*	0	18	0	0	0	0	0	0	0	0	5	18	D
15.	*Formicomus pedestris*	4	8	0	0	2	0	3	0	0	0	11	17	P
16.	*Lygaeus aequestris*	0	0	3	0	2	1	0	0	0	10	7	16	D
17.	*Bothynoderes punctiventris*	15	0	0	0	0	0	0	0	0	0	1	15	D
18.	*Coccinella 11punctata*	14	0	0	0	0	0	0	0	0	0	2	14	P

19.	*Pseudophonus griseus*	0	0	0	0	2	9	0	0	0	1	7	12	P
20.	*Cassida nebulosa*	8	3	0	0	1	2	0	1	0	0	7	15	D
21.	*Anthicus humeralis*	0	0	10	0	0	0	0	0	0	0	5	10	I
22.	*Oxythyrea funesta*	10	0	0	0	0	0	0	0	0	0	4	10	P
23.	*Harpalus tardus*	6	2	0	0	0	1	0	0	0	0	7	9	D
24.	*Podagrica malvae*	1	1	5	1	0	0	0	0	0	0	5	8	D
25.	*Aphthona euphorbiae*	0	0	4	0	0	0	2	0	1	0	5	7	D
26.	*Anisodactylus binotatus*	4	2	0	0	0	0	0	0	0	0	4	6	P
27.	*Harpalus calceatus*	3	1	0	0	1	0	0	0	0	0	4	5	P
28.	*Otiorrhynchus raucus*	2	0	0	0	2	1	0	0	0	0	5	5	D
29.	*Phyllobius pyri*	0	0	5	0	0	0	0	0	0	0	3	5	D
30.	*Calathus fuscipes*	1	0	0	0	0	0	0	0	0	3	3	4	P
31.	*Chromatoilus unilineatus*	1	0	1	0	0	1	0	0	1	0	4	4	P
32.	*Eremotes punctatus*	4	0	0	0	0	0	0	0	0	0	1	4	I
33.	*Pedinus femoralis*	0	4	0	0	0	0	0	0	0	0	3	4	I
34.	*Brachynus crepitans*	3	0	0	0	0	0	0	0	0	0	3	3	P
35.	*Elater nigerimus*	1	0	0	1	1	0	0	0	0	0	3	3	P
36.	*Ophonus azureus*	1	0	0	0	0	1	1	0	0	0	3	3	P
37.	*Oxytelus inustus*	3	0	0	0	0	0	0	0	0	0	1	3	P
38.	*Amara aenea*	2	0	0	0	0	0	0	0	0	0	2	2	P
39.	*Amara apricaria*	2	0	0	0	0	0	0	0	0	0	2	2	P
40.	*Amara crenata*	0	0	0	0	0	0	0	0	0	2	1	2	P
41.	*Anisodactylus signatus*	2	0	0	0	0	0	0	0	0	0	2	2	P
42.	*Ceutorhynchus macula alba*	0	1	0	0	1	0	0	0	0	0	2	2	D
43.	*Leistus fulvibarbis*	0	0	0	0	1	0	1	0	0	0	2	2	P
44.	*Metabletus truncatelus*	1	0	0	0	0	0	1	0	0	0	2	2	P
45.	*Phyllotreta atra*	0	1	0	0	0	0	0	0	1	0	2	2	D
46.	*Phyllotreta nemorum*	0	0	0	0	0	0	2	0	0	0	1	2	D
47.	*Acupalpus elegans*	0	1	0	0	0	0	0	0	0	0	1	1	P
48.	*Aglenus brunneus*	0	1	0	0	0	0	0	0	0	0	1	1	I
49.	*Aphodius granarius*	0	1	0	0	0	0	0	0	0	0	1	1	I
50.	*Baris artemisiae*	0	0	0	0	0	1	0	0	0	0	1	1	D
51.	*Bruchus pisorum*	0	1	0	0	0	0	0	1	0	0	2	2	D
52.	*Cantharis fusca*	0	0	0	1	0	0	0	0	0	0	1	1	P
53.	*Cantharis nigricans*	0	1	0	0	0	0	0	0	0	0	1	1	P
54.	*Carabus coriaceus*	0	0	0	0	1	0	0	0	0	0	1	1	P
55.	*Chrysopa perla*	0	0	0	0	0	0	0	0	1	0	1	1	P
56.	*Crypticus quisquilius*	0	0	0	0	0	1	0	0	0	0	1	1	I
57.	*Leptinotarsa decemlineata*	0	0	0	0	0	0	0	0	1	0	1	1	D
58.	*Longitarsus suturalis*	0	0	0	0	0	0	0	0	1	0	1	1	D
59.	*Malachius bipustulatus*	0	1	0	0	0	0	0	0	0	0	1	1	P
60.	*Meligethes aeneus*	1	0	0	0	0	0	0	0	0	0	1	1	D
61.	*Nebria jockischii*	0	0	1	0	0	0	0	0	0	0	1	1	P
62.	*Notaris maerkeli*	0	0	1	0	0	0	0	0	0	0	1	1	I
63.	*Ophonus rupicola*	0	0	1	0	0	0	0	0	0	0	1	1	P
64.	*Otiorrhynchus ovatus*	0	0	0	1	0	0	0	0	0	0	1	1	D
65.	*Otiorrhynchus pinastri*	0	0	1	0	0	0	0	0	0	0	1	1	D
66.	*Pentodon idiota*	1	0	0	0	0	0	0	0	0	0	1	1	D
67.	*Platynaspis luteorubra*	1	0	0	0	0	0	0	0	0	0	1	1	P
68.	*Pleurophorus caesus*	1	0	0	0	0	0	0	0	0	0	1	1	I
69.	*Pterostichus vernalis*	1	0	0	0	0	0	0	0	0	0	1	1	P
70.	*Rhinomias forticornis*	0	0	1	0	0	0	0	0	0	0	1	1	D
71.	*Rhyzophagus picipes*	0	0	0	0	0	0	1	0	0	0	1	1	I
72.	*Stenopterus rufus*	0	0	1	0	0	0	0	0	0	0	1	1	D
Total 72 species		431	323	203	75	80	183	51	88	22	171	120	1536	

***P=pradatory species, D=harmful species, I= indifferent species

Between May and September 2023, several insect species were collected in the pea crop using Barber traps. Among the most frequent species were *Dermestes laniarius* (277 specimens), *Messor structor* (274 specimens), and *Coccinella septempunctata* (201 specimens). Other species of interest included *Phalangium opilio* (151 specimens) and *Pseudophonus pubescens* (136 specimens). Additionally, rarer species such as *Anthicus floralis* and *Polydrusus amoenus* were collected, with a small number of specimens (20 and 18, respectively). Overall, various species of both beneficial and harmful insects were captured throughout the summer, having a significant impact.

Table 2. The situation regarding the calculation of ecological factors for each species during the period May -September, in the pea crop in 2023

No.	Name of species	No. traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Dermestes laniarius*	55	277	45,8	C2	18,0	D5	8,244	W4
2.	*Messor structor*	33	274	27,5	C2	17,8	D5	4,895	W3
3.	*Coccinella septempunctata*	34	201	28,3	C2	13,1	D5	3,707	W3
4.	*Phalangium opilio*	50	151	41,7	C2	9,8	D4	4,086	W3
5.	*Pseudophonus pubescens*	26	136	21,7	C1	8,9	D4	1,931	W3
6.	*Dermestes lardarius*	15	60	12,5	C1	3,9	D3	0,604	W2
7.	*Opatrum sabulosum*	25	59	20,8	C1	3,8	D3	0,790	W2
8.	*Pyrrhocoris apterus*	14	29	11,7	C1	1,9	D2	0,222	W2
9.	*Lixus cardui*	8	26	6,7	C1	1,7	D2	0,114	W2
10.	*Hipodamia variegata*	14	22	11,7	C1	1,4	D2	0,164	W2
11.	*Harpalus distinguendus*	9	20	7,5	C1	1,3	D2	0,097	W1
12.	*Sitona lineatus*	2	20	1,7	C1	1,3	D2	0,022	W1
13.	*Anthicus floralis*	5	20	4,2	C1	1,3	D2	0,055	W1
14.	*Polydrusus amoenus*	5	18	4,2	C1	1,3	D2	0,050	W1
15.	*Formicomus pedestris*	11	17	9,1	C1	1,2	D2	0,100	W2
16.	*Lygaeus aequestris*	7	16	5,8	C1	1,1	D1	0,058	W1
17.	*Bothynoderes punctiventris*	1	15	0,8	C1	1,0	D1	0,007	W1
18.	*Coccinella 11punctata*	2	14	1,7	C1	0,9	D1	0,015	W1
19.	*Pseudophonus griseus*	7	12	5,8	C1	0,9	D1	0,046	W1
20.	*Oxythyrea funesta*	4	10	3,3	C1	0,8	D1	0,019	W1
21.	*Anthicus humeralis*	5	10	4,2	C1	0,6	D1	0,025	W1
22.	*Harpalus tardus*	7	9	5,8	C1	0,6	D1	0,035	W1
23.	*Cassida nebulosa*	3	8	2,5	C1	0,6	D1	0,012	W1
24.	*Podagrica malvae*	5	8	4,2	C1	0,5	D1	0,021	W1
25.	*Aphthona euphorbiae*	5	7	4,2	C1	0,5	D1	0,016	W1
26.	*Anisodactylus binotatus*	4	6	3,3	C1	0,4	D1	0,010	W1
27.	*Harpalus calceatus*	4	5	3,3	C1	0,3	D1	0,010	W1
28.	*Otiorrhynchus raucus*	5	5	4,2	C1	0,3	D1	0,013	W1
29.	*Phyllobius pyri*	3	5	2,5	C1	0,3	D1	0,007	W1
30.	*Calathus fuscipes*	3	4	2,5	C1	0,3	D1	0,007	W1
31.	*Eremotes punctatus*	1	4	0,8	C1	0,3	D1	0,002	W1
32.	*Chromatoilus*	4	4	3,3	C1	0,3	D1	0,010	W1
33.	*Pedinus femoralis*	3	4	2,5	C1	0,3	D1	0,007	W1
34.	*Brachynus crepitans*	3	3	2,5	C1	0,2	D1	0,005	W1
35.	*Elater nigerimus*	3	3	2,5	C1	0,2	D1	0,005	W1
36.	*Ophonus azureus*	3	3	2,5	C1	0,2	D1	0,005	W1
37.	*Oxytelus inustus*	1	3	0,8	C1	0,2	D1	0,002	W1
38.	*Cassida nebulosa*	3	3	2,5	C1	0,2	D1	0,005	W1
39.	*Amara aenea*	2	2	1,7	C1	0,1	D1	0,002	W1
40.	*Amara apricaria*	2	2	1,7	C1	0,1	D1	0,002	W1
41.	*Anisodactylus signatus*	2	2	1,7	C1	0,1	D1	0,002	W1
42.	*Metabletus truncatelus*	2	2	1,7	C1	0,1	D1	0,002	W1
43.	*Ceutorhynchus macula alba*	2	2	1,7	C1	0,1	D1	0,002	W1
44.	*Phyllotreta atra*	2	2	1,7	C1	0,1	D1	0,002	W1
45.	*Phyllotreta nemorum*	1	2	0,8	C1	0,1	D1	0,001	W1
46.	*Amara crenata*	1	2	0,8	C1	0,1	D1	0,001	W1
47.	*Meligethes aeneus*	1	1	0,8	C1	0,1	D1	0,001	W1
48.	*Pentodon idiota*	1	1	0,8	C1	0,1	D1	0,001	W1
49.	*Platynaspis luteorubra*	1	1	0,8	C1	0,1	D1	0,001	W1
50.	*Pleurophorus caesus*	1	1	0,8	C1	0,1	D1	0,001	W1
51.	*Pterostichus vernalis*	1	1	0,8	C1	0,1	D1	0,001	W1
52.	*Acupalpus elegans*	1	1	0,8	C1	0,1	D1	0,001	W1
53.	*Aglenus brunneus*	1	1	0,8	C1	0,1	D1	0,001	W1
54.	*Aphodius granarius*	1	1	0,8	C1	0,1	D1	0,001	W1
55.	*Bruchus pisorum*	1	1	0,8	C1	0,1	D1	0,001	W1
56.	*Cantharis nigricans*	1	1	0,8	C1	0,1	D1	0,001	W1
57.	*Malachius bipustulatus*	1	1	0,8	C1	0,1	D1	0,001	W1
58.	*Nebria jockischii*	1	1	0,8	C1	0,1	D1	0,001	W1
59.	*Notaris maerkeli*	1	1	0,8	C1	0,1	D1	0,001	W1
60.	*Ophonus rupicola*	1	1	0,8	C1	0,1	D1	0,001	W1
61.	*Otiorrhynchus pinastri*	1	1	0,8	C1	0,1	D1	0,001	W1
62.	*Rhinomias forticornis*	1	1	0,8	C1	0,1	D1	0,001	W1
63.	*Stenopterus rufus*	1	1	0,8	C1	0,1	D1	0,001	W1
64.	*Cantharis fusca*	1	1	0,8	C1	0,1	D1	0,001	W1
65.	*Otiorrhynchus ovatus*	1	1	0,8	C1	0,1	D1	0,001	W1
66.	*Carabus coriaceus*	1	1	0,8	C1	0,1	D1	0,001	W1
67.	*Leistus fulvibarbis*	1	1	0,8	C1	0,1	D1	0,001	W1
68.	*Baris artemisiae*	1	1	0,8	C1	0,1	D1	0,001	W1
69.	*Crypticus quisquilius*	1	1	0,8	C1	0,1	D1	0,001	W1
70.	*Leistus fulvibarbis*	1	1	0,8	C1	0,1	D1	0,001	W1

71.	*Rhyzophagus picipes*	1	1	0,8	C1	0,1	D1	0,001	W1
72.	*Bruchus pisorum*	1	1	0,8	C1	0,1	D1	0,001	W1
73.	*Leptinotarsa decemlineata*	1	1	0,8	C1	0,1	D1	0,001	W1
74.	*Longitarsus suturalis*	1	1	0,8	C1	0,1	D1	0,001	W1
75.	*Chrysopa perla*	1	1	0,8	C1	0,1	D1	0,001	W1
Total 75 species		120	1536						

The data in Table 2 refer to the ecological factors of the species collected between May and September 2023 in the pea crop, considering parameters such as Abundance, Constancy, Dominance, and Ecological Significance Index. The most abundant species are *Dermestes laniarius, Messor structor,* and *Coccinella septempunctata*, with significant abundance values (over 200 specimens). Most species have a high constancy (C2), indicating frequent presence. Constancy (C1-C4) ranges from accidental species (C1, 1-2%) such as *Bothynoderes punctiventris* and *Amara aenea*, to constant species (C3, 50.1-75%), such as *Phalangium opilio*. Species with high constancy, such as *Dermestes laniarius*, are essential in the ecosystem. Species that appear very rarely in the area or within a limited period, with low presence, are considered accidental with sporadic occurrence.

Dominance (D1-D5) reflects the presence and influence of species. Subrecent species (D1), such as *Amara apricaria*, have low presence, while *Dermestes laniarius* and *Messor structor* are eudominant (D5), having a major impact on the community.

The Ecological Significance Index shows variable adaptability (W1-W5), ranging from accidental species with low adaptability (W1), such as *Phyllobius pyri*, to highly adaptable species (W4), such as *Dermestes laniarius*, which are well integrated into the ecosystem.

Table 3. The situation regarding the number of collections in which each species was collected during the period May-September, 2024

No.	Name of species	Harvest						No. traps	Total samples	Food types ***
		I	II	III	IV	V	VI			
1.	*Coccinella septempunctata*	161	41	65	1	0	0	37	278	P
2.	*Opatrum sabulosum*	172	3	1	0	0	0	15	274	D
3.	*Phalangium opilio*	29	33	66	12	16	6	44	201	P
4.	*Pseudophonus pubescens*	1	0	8	11	47	85	31	151	P
5.	*Messor structor*	11	1	40	32	53	1	20	136	P
6.	*Dermestes laniarius*	10	7	47	3	5	2	33	60	P
7.	*Hipodamia variegatta*	38	11	23	0	0	0	19	57	P
8.	*Lygaeus aequsris*	2	8	12	7	8	11	22	27	I
9.	*Sitona lineatus*	26	0	0	0	0	0	6	26	D
10.	*Crypticus quisquilius*	18	6	0	0	0	0	11	22	D
11.	*Cleonus punctiger*	0	0	21	0	0	0	1	20	D
12.	*Pedinus femoralis*	8	0	9	0	0	0	7	20	D
13.	*Podonta nigrita*	0	0	14	1	0	0	8	20	P
14.	*Podagrica malvae*	0	12	1	2	0	3	11	18	I
15.	*Formicomus pedestris*	9	0	0	1	2	0	6	17	P
16.	*Pedinus femoralis*	0	10	0	0	0	0	13	16	I
17.	*Harpalus distinguendus*	1	0	5	0	0	1	6	15	P
18.	*Chromatoiulus unilineatus*	5	0	1	0	0	1	3	14	P
19.	*Otiorhynchus sulcatus*	6	0	0	0	0	0	4	12	I
20.	*Anisodactylus binotatus*	0	1	5	0	0	0	3	15	P
21.	*Pseudophonus griseus*	0	0	0	0	0	5	3	10	P
22.	*Harpalus tardus*	1	1	0	0	1	1	4	10	P
23.	*Hister purpurascens*	1	2	1	0	0	0	4	9	P
24.	*Anthicus floralis*	2	0	0	0	2	0	2	8	I
25.	*Adalia bipunctata*	0	0	4	0	0	0	2	7	P
26.	*Tanymecus palliatus*	1	1	1	0	0	0	3	6	D
27.	*Botynoderes punctiventris*	0	2	0	1	0	0	3	5	D
28.	*Ophonus azureus*	0	1	1	0	0	0	2	5	P
29.	*Chrysomella menthastri*	0	0	2	0	0	0	2	5	I
30.	*Corymbites affinis*	0	0	2	0	0	0	2	4	D
31.	*Longitarsus anchusae*	0	0	0	1	1	0	2	4	D
32.	*Aleochara laevigata*	1	0	0	0	0	0	1	4	P
33.	*Aleochara moereus*	1	0	0	0	0	0	1	4	P
34.	*Calathus fuscipes*	1	0	0	0	0	0	1	3	P
35.	*Cantharis fusca*	1	0	0	0	0	0	1	3	P
36.	*Hister sepulchralis*	1	0	0	0	0	0	1	3	P
37.	*Metabletus foveatus*	1	0	0	0	0	0	1	3	P

38.	*Phyllobius pyri*	1	0	0	0	0	0	1	2	D
39.	*Tanymecus dilaticolis*	1	0	0	0	0	0	1	2	D
40.	*Valgus hemipterus*	1	0	0	0	0	0	1	2	I
41.	*Amara familiaris*	0	1	0	0	0	0	1	2	P
42.	*Bruchus pisorum*	0	1	0	0	0	0	1	2	D
43.	*Crepidodera transversa*	1	0	0	0	0	0	1	2	D
44.	*Formicomus gracilis*	1	0	0	0	0	0	1	2	P
45.	*Oedemera virescens*	0	1	0	0	0	0	1	2	D
46.	*Ophonus puncticolis*	0	1	0	0	0	0	1	2	P
47.	*Tenebroides mauritanicus*	0	1	0	0	0	0	1	1	D
48.	*Agriotes lineatus*	0	0	1	0	0	0	1	1	D
49.	*Harpalus calceatus*	0	0	1	0	0	0	1	1	P
50.	*Necrophorus vespillo*	0	0	1	0	0	0	1	1	I
51.	*Pentodon idiota*	0	0	1	0	0	0	1	2	D
52.	*Polydrusus confluens*	0	0	1	0	0	0	1	1	D
53.	*Agriotes ustulatus*	0	0	0	1	0	0	1	1	D
54.	*Amara aenea*	0	0	0	1	0	0	1	1	P
55.	*Coccidula scutellata*	0	0	0	1	0	0	1	1	P
56.	*Elater elongatulus*	0	0	0	1	0	0	1	1	D
57.	*Elater erythtogonus*	0	0	0	1	0	0	1	1	D
58.	*Ptreyngium crenatum*	0	0	0	1	0	0	1	1	I
59.	*Amara crenata*	0	0	0	0	1	0	1	1	P
60.	*Mecinus janthinus*	0	0	0	0	1	0	1	1	D
61.	*Microlestes maurus*	0	0	0	0	1	0	1	1	P
62.	*Soronia punctatissima*	0	0	0	0	1	0	1	1	D
63.	*Amara aenea*	0	0	0	0	0	1	1	1	P
64.	*Procerus tibialis*	0	0	0	0	0	1	1	1	P
Total	64 species	513	145	334	78	139	118	72	1323	

***P=pradatory species, D=harmful species, I= indifferent species

The data summarized in Table 3 presents the distribution of species based on the number of collections made between May and September 2024. It is observed that species such as *Coccinella septempunctata, Opatrum sabulosum*, and *Phalangium opilio* are the most frequent, with a large number of specimens collected. On the other hand, other species such as *Pseudophonus pubescens* and *Messor structor* are less abundant, but still significant. Occasional species, such as *Cleonus punctiger* and *Podagrica malvae*, only appear in specific months.

Out of the total 1,323 specimens collected, 278 belong to *Coccinella septempunctata*, followed by *Opatrum sabulosum, Phalangium opilio, Pseudophonus pubescens,* and *Messor structor*, all with over 100 specimens. The species vary depending on their preferred food types (*P, D, I*), and the total of 64 species reflects an important ecological diversity during this period.

Tracking the dynamics of the collected species shows that 32 out of the 64 species collected recorded only a single specimen, categorizing them as occasional species. This variability indicates a large diversity of species depending on the habitat and environmental conditions.

Table 4. The situation regarding the calculation of ecological factors for each species during the period May-September, in the pea crop in 2024

No.	Name of species	No. traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Phalangium opilio*	44	162	61,2	C3	12,2	D5	7,466	W4
2.	*Coccinella septempunctata*	37	268	51,4	C3	20,3	D5	10,434	W5
3.	*Dermestes laniarius*	33	74	45,8	C2	5,6	D4	2,565	W3
4.	*Pseudophonus pubescens*	31	152	43,1	C2	11,5	D5	4,956	W3
5.	*Lygaeus aequsris*	22	48	30,5	C2	3,6	D3	1,102	W3
6.	*Messor structor*	20	138	27,8	C2	10,4	D5	2,891	W3
7.	*Hipodamia variegatta*	19	72	26,4	C2	5,4	D4	1,425	W3
8.	*Opatrum sabulosum*	15	176	20,8	C1	13,3	D5	2,766	W3
9.	*Pedinus femoralis*	13	10	18,1	C1	0,8	D1	0,145	W2
10.	*Podagrica malvae*	11	14	15,3	C1	1,1	D2	0,168	W2
11.	*Crypticus quisquilius*	11	24	15,3	C1	1,8	D2	0,275	W2
12.	*Podonta nigrita*	8	15	11,2	C1	1,1	D2	0,123	W2
13.	*Pedinus femoralis*	7	17	9,7	C1	1,3	D2	0,126	W2
14.	*Harpalus distinguendus*	6	7	8,3	C1	0,5	D1	0,041	W1
15.	*Formicomus pedestris*	6	12	8,3	C1	0,9	D1	0,074	W1
16.	*Sitona lineatus*	6	26	8,3	C1	1,9	D2	0,158	W2

17.	*Harpalus tardus*	4	4	5,6	C1	0,3	D1	0,017	W1
18.	*Hister purpurascens*	4	4	5,6	C1	0,3	D1	0,017	W1
19.	*Otiorhynchus sulcatus*	4	6	5,6	C1	0,5	D1	0,028	W1
20.	*Tanymecus palliatus*	3	3	4,1	C1	0,2	D1	0,008	W1
21.	*Botynoderes punctiventris*	3	3	4,1	C1	0,2	D1	0,008	W1
22.	*Pseudophonus griseus*	3	5	4,1	C1	0,4	D1	0,016	W1
23.	*Anisodactylus binotatus*	3	6	4,1	C1	0,5	D1	0,020	W1
24.	*Chromatoiulus unilineatus*	3	7	4,1	C1	0,5	D1	0,020	W1
25.	*Ophonus azureus*	2	2	2,8	C1	0,2	D1	0,006	W1
26.	*Chrysomella menthastri*	2	2	2,8	C1	0,2	D1	0,006	W1
27.	*Corymbites affinis*	2	2	2,8	C1	0,2	D1	0,006	W1
28.	*Longitarsus anchusae*	2	2	2,8	C1	0,2	D1	0,006	W1
29.	*Anthicus floralis*	2	4	2,8	C1	0,3	D1	0,008	W1
30.	*Adalia bipunctata*	2	4	2,8	C1	0,3	D1	0,008	W1
31.	*Aleochara laevigata*	1	1	1,4	C1	0,1	D1	0,001	W1
32.	*Aleochara moereus*	1	1	1,4	C1	0,1	D1	0,001	W1
33.	*Calathus fuscipes*	1	1	1,4	C1	0,1	D1	0,001	W1
34.	*Cantharis fusca*	1	1	1,4	C1	0,1	D1	0,001	W1
35.	*Hister sepulchralis*	1	1	1,4	C1	0,1	D1	0,001	W1
36.	*Metabletus foveatus*	1	1	1,4	C1	0,1	D1	0,001	W1
37.	*Phyllobius pyri*	1	1	1,4	C1	0,1	D1	0,001	W1
38.	*Tanymecus dilaticolis*	1	1	1,4	C1	0,1	D1	0,001	W1
39.	*Valgus hemipterus*	1	1	1,4	C1	0,1	D1	0,001	W1
40.	*Amara familiaris*	1	1	1,4	C1	0,1	D1	0,001	W1
41.	*Bruchus pisorum*	1	1	1,4	C1	0,1	D1	0,001	W1
42.	*Crepidodera transversa*	1	1	1,4	C1	0,1	D1	0,001	W1
43.	*Formicomus gracilis*	1	1	1,4	C1	0,1	D1	0,001	W1
44.	*Oedemera virescens*	1	1	1,4	C1	0,1	D1	0,001	W1
45.	*Ophonus puncticolis*	1	1	1,4	C1	0,1	D1	0,001	W1
46.	*Tenebroides mauritanicus*	1	1	1,4	C1	0,1	D1	0,001	W1
47.	*Agriotes lineatus*	1	1	1,4	C1	0,1	D1	0,001	W1
48.	*Harpalus calceatus*	1	1	1,4	C1	0,1	D1	0,001	W1
49.	*Necrophorus vespillo*	1	1	1,4	C1	0,1	D1	0,001	W1
50.	*Pentodon idiota*	1	1	1,4	C1	0,1	D1	0,001	W1
51.	*Polydrusus confluens*	1	1	1,4	C1	0,1	D1	0,001	W1
52.	*Agriotes ustulatus*	1	1	1,4	C1	0,1	D1	0,001	W1
53.	*Amara aenea*	1	1	1,4	C1	0,1	D1	0,001	W1
54.	*Coccidula scutellata*	1	1	1,4	C1	0,1	D1	0,001	W1
55.	*Elater elongatulus*	1	1	1,4	C1	0,1	D1	0,001	W1
56.	*Elater erythtogonus*	1	1	1,4	C1	0,1	D1	0,001	W1
57.	*Ptreyngium crenatum*	1	1	1,4	C1	0,1	D1	0,001	W1
58.	*Amara crenata*	1	1	1,4	C1	0,1	D1	0,001	W1
59.	*Mecinus janthinus*	1	1	1,4	C1	0,1	D1	0,001	W1
60.	*Microlestes maurus*	1	1	1,4	C1	0,1	D1	0,001	W1
61.	*Soronia punctatissima*	1	1	1,4	C1	0,1	D1	0,001	W1
62.	*Amara aenea*	1	1	1,4	C1	0,1	D1	0,001	W1
63.	*Procerus tibialis*	1	1	1,4	C1	0,1	D1	0,001	W1
64.	*Cleonus punctiger*	1	21	1,4	C1	0,1	D2	0,022	W1
Total 64 species		72	1323						

Ecological analysis for May-September 2024 (Table 4) reveals that *Coccinella septempunctata* and *Phalangium opilio* are the most dominant species, exhibiting high frequency, constancy, and abundance, and playing a pivotal role in shaping the ecosystem, while species like *Dermestes laniarius, Pseudophonus pubescens*, and *Messor structor*, though important, are less dominant. Over two years of study, the epigeic fauna in the pea crop is primarily composed of beneficial species, followed by harmful ones, with indifferent species representing a relatively small proportion in the overall community structure.

Between 2023 and 2024 (Table 5, Figure 1), the total number of specimens collected decreased from 1,536 to 1,323, with a significant reduction in predator species (81% → 72%) and an increase in harmful species (16% → 22%). This change suggests a potential ecological imbalance that could favor the proliferation of pests.

The increase in indifferent species (3% → 6%) indicates a modification of the habitat. The decline in predator species may be caused by climatic or agricultural factors, requiring biodiversity protection measures to maintain balance in this pea crop.

Table 5. Centralization of the data obtained over the two years of research regarding the feeding spectrum of the collected species

No	Food type	2023		2024	
		No. of samples	%	No. of samples	%
1	Pradatory species	1246	81	948	72
2	Harmful species	246	16	288	22
3	Indifferent species	44	3	87	6
Total samples		1536	100	1323	100

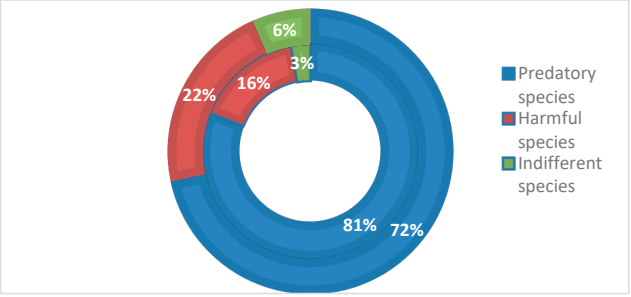


Figure 1. Graphical representation of the epigeic fauna structure based on the type of food in 2023-2024 period

CONCLUSIONS

The structure of the epigeic arthropod fauna has changed over the course of the observations. Between 2023 and 2024, there was a decrease in the total number of species collected, with a significant reduction in predator species and an increase in harmful species, indicating a potential ecological imbalance.

Dominant species from the beneficial fauna, such as *Coccinella septempunctata* and *Phalangium opilio*, were the most prevalent, showing high constancy and abundance, which gives them an important role in maintaining the balnce of the ecosystem.

The significance of eudominant species, such as *Dermestes laniarius*, *Pseudophonus pubescens*, and *Messor structor*, is also noteworthy, as they have a significant influence on the ecosystem, although they are not as dominant as *Coccinella septempunctata*, also contributing to ecological balance.

The increase in the number of harmful species from 16% to 22% could affect the pea crop, requiring additional pest control and protection measures to prevent damage to the production.

Analyzing the data collected during the two years of observation, we can see a decrease in the number of usedfull species and their specimens, as well as an increase in indifferent species, suggesting a modification of the habitat. Therefore, actions to protect the fauna are necessary to maintain a stable ecological balance.

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CONSERVATION CHALLENGES FOR *LIPARIS LOESELII* (L.) RICH.: A CRITICALLY ENDANGERED ORCHID IN ROMANIA

Constantin-Ciprian BÎRSAN[1], Marilena ONETE[1], Sorin ȘTEFĂNUȚ[1], Roxana-Georgiana NICOARĂ[1], Tiberiu SAHLEAN[1], Attila MÁTIS[2], Anna SZABÓ[2], Simona MIHĂILESCU[1]

[1]Institute of Biology Bucharest of Romanian Academy,
296 Splaiul Independentei, District 6, Bucharest, Romania
[2]Babeş-Bolyai University, Faculty of Biology and Geology,
5-7 Clinicilor Street, Cluj-Napoca, Romania

Corresponding author email: marilena.onete@ibiol.ro

Abstract

Liparis loeselii is a rare and highly threatened orchid species in Europe. With a European conservation status of Near Threatened (IUCN), this species is strictly protected under many EU and national regulations. It is present in 475 Natura 2000 sites across Europe, with only 10 sites from Romania. In Romania, L. loeselii is classified as Critically Endangered (CR), marking the southeastern limit of its distribution range. We monitored the species in the context of Article 17 of the Habitats Directive for EU specific reporting of Romania. The species inhabits hygrophilous, eutrophic fens characterized by calcium-rich, neutral to alkaline pH conditions, cold water, and black soil with a humus-turf layer overlaying a silt horizon. These habitats are increasingly threatened by drainage and fragmentation, leading to significant population declines. Historical records indicate that many populations previously cited in literature have not been confirmed in recent surveys, suggesting a critical need for updated assessments and conservation measures. This study underscores the urgency of habitat protection and restoration to prevent further decline and ensure the survival of L. loeselii within its natural range.

Key words*: conservation status, extinction threats, fen orchid, Liparis loeselii, protected species.*

INTRODUCTION

Liparis loeselii (L.) Rich., commonly known as fen orchid, is a rare and highly threatened orchid species in Europe, characterized by its preference for wetland habitats. It is listed as Near Threatened (NT) under the IUCN Red List (Bilz, 2011) and is strictly protected by various EU and national regulations, including the Bern Convention (1979) and the EU Habitats Directive (92/43/EEC). The species serves as an ecological indicator for fen ecosystems due to its strict habitat requirements, making it a subject of conservation interest (Oostermeijer & Hartman, 2014).

According to EUNIS, *L. loeselii* is found in 475 Natura 2000 sites across Europe, yet its presence in Romania is limited to only 10 sites, indicating a highly fragmented and vulnerable population. This orchid predominantly inhabits calcium-rich, neutral to alkaline fens, where cold groundwater maintains stable hydrological conditions. The substrates supporting this species are characterized by humus-turf layers over silt horizons, essential for the development of its seeds, which rely on mycorrhizal associations for germination (Błońska et al., 2016).

Over recent decades, populations of *L. loeselii* have declined significantly due to habitat destruction (Pillon et al., 2007; Błońska et al., 2016; Szatmari, 2022; Andersen et al., 2025). The primary threats include:

- drainage and hydrological alterations, leading to habitat desiccation and seedling mortality;
- habitat fragmentation, reducing genetic flow and increasing local extinction risks;
- expansion of tall perennials such as *Phragmites australis* and *Typha latifolia*, which compete for space and resources;
- climate change impacts, particularly shifts in precipitation patterns and increasing temperatures, which alter fen hydrology and soil conditions.

Given these conservation challenges, this study aims to provide an updated assessment of the

current population status of *L. loeselii* in Romania under the framework of Article 17 of the EU Habitats Directive.

MATERIALS AND METHODS

Analysing the scientific articles, reports, and management plans we developed a database used for all the further specific analysis.

In the *Habitats Directive* (92/43/EEC), monitoring of Annex II and Annex IV species is required in Article 11 and into the Article 17 requires reporting every six years, for each Member State of the EU. Member States shall undertake surveillance of the conservation status of the species with particular regard to priority species. Measures taken pursuant to the *Habitats Directive* shall be designed to maintain or restore, at favourable conservation status for species of wild flora of Community interest.

The Natura 2000 species code for *Liparis loeselii* (L.) Rich. is **1903**. In Romania the species is present in two biogeographical regions: ALP = Alpine and CON = Continental. In the last 12 years, all Member States, including Romania, has reported two times to the Commission: in 2013 (for the evaluation period 2007-2012) and 2019 (for the evaluation period 2013-2018), based on every 6 years of the assessed conservation status for habitats of community interest (S.O.P., 2019; L.I.O.P., 2023). For the next report (deadline 31 July 2025), was approved in 2023 the reporting format on species types (DG Environment, 2023).

Within these framework projects, carried out between 2011-2023, the *L. loeselii* population was inventoried in Romania and the conservation status of this species was evaluated (Mihăilescu et al., 2015).

The species identification was carried out in the field, no specimens were collected.

The concept of favourable reference values is derived from definitions in the *Habitats Directive* (DG Environment, 2023). Overall assessment of conservation status uses four categories: "favourable" (FV), "unfavourable-inadequate" (U1), "unfavourable-bad" (U2) and "unknown" (XX), based on the evaluation matrix for assessing conservation status for a species (Table 1).

Table 1. Overall assessment of the conservation status (CS)

Status of parameters	All favourable or few favourable + one unknown	One or more inadequate, but no bad	One or more bad	Two or more unknown +favourable or all unknown
Overall assessment of CS	Favourable (FV)	unfavourable-inadequate (U1)	unfavourable-bad (U2)	Unknown (XX)

RESULTS AND DISCUSSIONS

Species Occurrence and Distribution

A total of 66 occurrences of *L. loeselii* (Figures 1 and 2) were compiled and documented in a database. These include 12 herbarium records from the collections of Babeș-Bolyai University and Mureș County Museum, 23 occurrences from scientific publications, and 31 records from monitoring efforts under Article 17 of the EU Habitats Directive.



Figure 1. *Liparis loeselii* (L.) Rich. in the Hărman eutrophic fens. The individual is shown in the fruiting stage, with several developing seed capsules visible at the top of the inflorescence. The plant is rooted in a wet, moss-covered substrate, characteristic of alkaline fen habitats, and surrounded by typical fen vegetation such as *Schoenus nigricans*. This stage reflects the species' successful reproductive cycle in situ, despite its known vulnerability (photo by Sorin Ștefănuț, 11.08.2016)

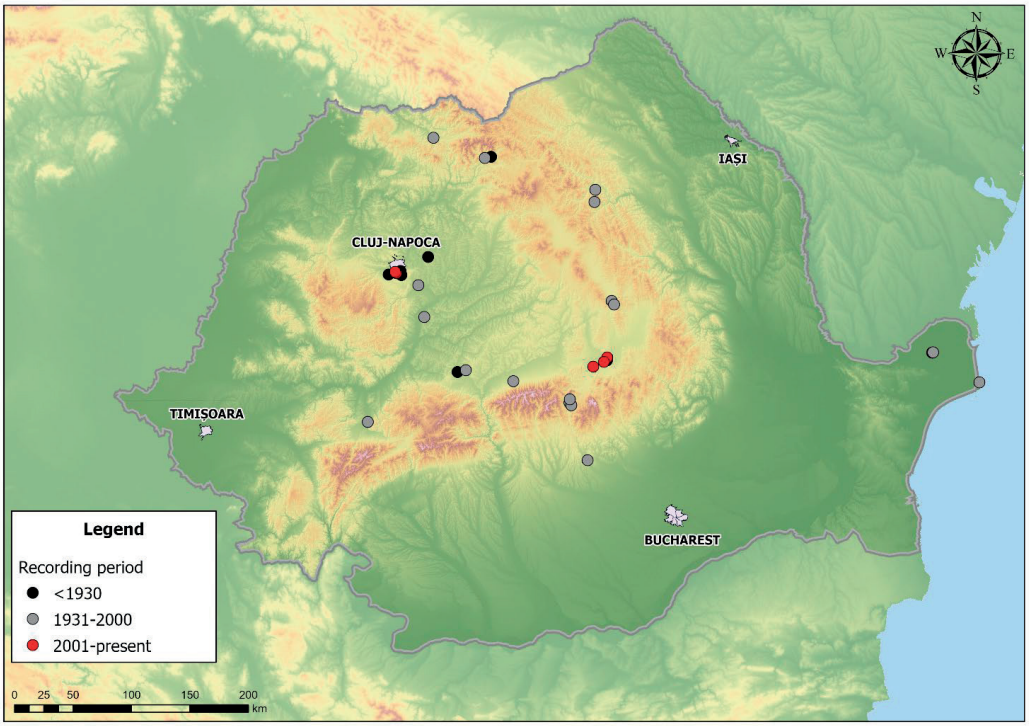


Figure 2. The distribution of *Liparis loeselii* (L.) Rich., a rare and protected orchid species, across various regions. The map highlights the historical and current presence of the species, providing valuable insights into its ecological preferences and conservation status

The historical presence of *L. loeselii* is supported by herbarium specimens dating as far back as 1881 and 1915, while scientific interest peaked in the 1970s with the publication of volumes on Romania's flora. Research has continued at a fluctuating pace, with a steady interest observed from the 1980s to 2016.

According to the EUNIS, the species is currently documented in 10 Natura 2000 sites, including Apuseni, Ceahlău, Dealul Cetății Lempeș-Mlaștina Hărman, Făgetul Clujului-Valea Morii, Munții Făgăraș, Munții Maramureșului, Pădurea și mlaștinile eutrofe de la Prejmer, Piatra Craiului, Sărăturile Ocna Veche, and Vânători-Neamț. Additionally, the Băgău site (AB County) is mentioned in the Romanian Natura 2000 catalog (Brînzan et al., 2013).

According to Witkowski et al. (2003), the species is present in eight divisions of the Carpathians, including Maramureș Mountains, Rodnei Mountains, Ceahlău, Harghita Mountains, Brașov Depression, Poiana Ruscă, Făgăraș Mountains, and the Transylvanian Plateau. These divisions overlap totally or partially with the Natura 2000 sites presented above.

Herbarium records from 1857-1931 document the species in Rodna Nouă (BN County), Hărman (BV County), Ocna Sibiului (SB County), Șuătru, Feleacu, Vîlcele, and Cluj (CJ County), as well as in the Danube Delta (TL Country).

Between 1930 and 2004, *L. loeselii* was reported in scientific publications from multiple locations, including Tăul fără fund de la Băgău (AB County); Pietricica Mountains, Piatra Craiului Mountains and Dâmbovicioarei Valley (AG County); Rodna (BN County); Stupini and Hărman (BV County); Morii Valley, Făgetul Clujului and Băile Sărate de la Turda (CJ County); Tătărani (DB County); between Covragiu and Subcetate (HD County); Puciosul Mountains and Lake Sfânta Ana (HR County); Dragomirești and Dumbrava (MM County); Răpciune and Ceahlău Mountains (NT County); Arpașu de Sus, Ocna Sibiului and Șura Mare (SB County); in the Danube Delta and Sfântu

Gheorghe (TL Country) (Paucă, 1972; Dihoru & Negrean, 2009).
However, since 2000, new records exist for only four locations: Stupini, Hărman, Prejmer (BV County), and Făgetul Clujului, Morii Vally (CJ County) (Figure 2).

Altitudinal range
The altitudinal distribution of *L. loeselii* in Romania (Figure 3) spans a significant range, from 5 m a.s.l. (Danube Delta) to 1260 m a.s.l. (Piatra Craiului Mountains). The mean elevation of occurrences is approximately 606 m, with a median of 640 m, suggesting a preference for mid-altitude habitats. However, the presence of multiple records clustered around 640 m highlights a potential ecological optimum in rich fen environments within this elevation range (Szatmari, 2022; Pop, 1960).

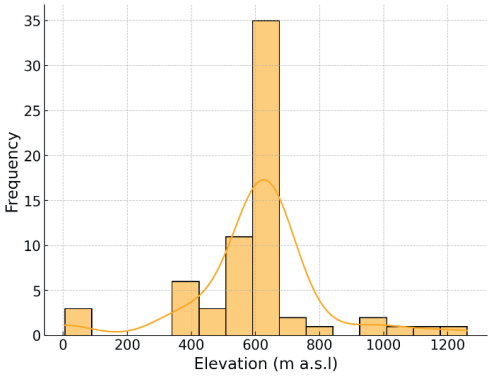


Figure 3. Altitudinal range of *Liparis loeselii* (L.) Rich. in Romania. The horizontal line represents the most frequent elevation range where the species has been recorded

Population Trends
Stupini eutrophic fen was first documented floristically by Diaconeasa in 1957. Subsequent studies by Morariu and Negrus in 1970 highlighted the presence of *Carex lasiocarpa, Drosera anglica*, and *L. loeselii* as well-represented species in the area (Parascan & Danciu, 1976). *L. loeselii* was first identified in Stupini by Pop in 1960, marking its initial recognition in this habitat.
In 2013, the population of *L. loeselii* was estimated at approximately 15 flowering individuals, located within a 9-hectare remnant of the once extensive eutrophic fen. The area has been significantly affected by drainage activities and the conversion of wetlands into agricultural land, leading to habitat degradation and increased vulnerability of the remaining population. However, a reassessment conducted in July 2022 revealed no detectable specimens of the species. This absence it might be a consequence of the assessment activities done after the optimal development of the plant or is likely attributed to a combination of factors, including the severe drought conditions experienced in 2022, ongoing drainage activities, and upstream water abstraction from the Lauterbach tributary.
The species is known to thrive in highly specific ecological conditions, almost exclusively inhabiting highly wet floating mats within the Carpathian-Pannonian region (Illyés, 2006). *L. loeselii* reliance on such specialized habitats makes it particularly vulnerable to changes in hydrology and water availability (Andersen et al., 2025).
Hărman: The flora of the Hărman eutrophic fens was first studied by Moesz in 1905, with subsequent investigations conducted by Pop in 1960.



Figure 4. *Liparis loeselii* (L.) Rich. in the Hărman eutrophic fens (photo by Sorin Ștefănuț, 08.07.2011)

In the summer months of July and August 1965, as well as in 1966, several specimens of *L. loeselii* were observed flowering and fruiting, as documented by Ularu and Danciu (1968).
In 2011 were found eight individual plants with flowers (Figure 4).
In 2014, as part of a research project, *L. loeselii* was once again identified in the Hărman fens, alongside *Ligularia sibirica*. The discovered specimens were protected and monitored to ensure their conservation. Proposed conservation measures included efforts to propagate and preserve *L. loeselii* to enhance its population and ensure its long-term persistence in the area (Miriea et al., 2014).
The eutrophic fens of Hărman are a highly vulnerable wetland habitat, characterized by a mosaic of dry and green vegetation patches, likely influenced by seasonal hydrological fluctuations and anthropogenic pressures.
In recent surveys assessed in 2016, the population counted eleven individuals.
Prejmer: The flora of the Prejmer eutrophic fens was previously studied by Pop (1960), Morariu (1966), and Parascan & Danciu (1976); however, *L. loeselii* was not reported in these early investigations.
The species was rediscovered in 2013, with a single individual identified during the project "Environmental restoration and support of natural processes in the forests and eutrophic marshes from Prejmer and Hărman", implemented by the Carpathian Foundation (BV County). A subsequent reassessment in July 2013 confirmed the species' presence. According to the final report of the project, 15 individuals were observed in 2015, followed by 20 individuals recorded in both 2016 and 2017, indicating a potentially stable but small population.

Făgetul Clujului-Valea Morii: The presence of *L. loeselii* (Figure 5) in the Făgetul Clujului-Valea Morii area has been documented since the early floristic studies conducted by Pop (1960) and Pop et al. (1962). The species was reconfirmed in 1998 by Ruprecht, who reported the rediscovery of both *L. loeselii* and *Bistorta officinalis*, species previously considered extinct from this site. *L. loeselii* was observed in two distinct zones of the fen, particularly near spring sources that maintain the moisture required for the orchid's survival. Individuals were recorded in small clusters of 4-5 plants, suggesting a limited and potentially vulnerable population.
The site holds high ecological value, acting as a refuge for glacial relicts and rare plant species, including *Ligularia sibirica, Tofieldia calyculata*, *Swertia perennis, Carex appropinquata, Ophioglossum vulgatum*, and *Dactylorhiza traunsteineri* (Cristea, 2014; Szatmari, 2022; Bartók et al., 2018).



Figure 5. *Liparis loeselii* (L.) Rich. in the Valea Morii fens, captured during its flowering phase. The plant is shown in close proximity to a human hand, providing a clear scale reference that highlights the delicate and diminutive size of this orchid species. This morphological detail emphasizes the species' vulnerability to habitat disturbance and the difficulty of detection during field surveys (photo by Attila Mátis, 23.06.2017)

More recent monitoring between 2011 and 2018 confirmed that the largest known Romanian population of *L. loeselii* exists in this area, with 184 individuals within the ROSCI0074 Natura 2000 site, and an additional 86 individuals located just outside the protected boundaries.
Population size measured in number of individuals is estimated between 200-800.

Habitat Preferences and Conservation Status
The species thrives in hygrophilous, eutrophic fens with calcium-rich, neutral to alkaline pH soils, cold water sources, and black soil layers over silt horizons (Dihoru and Negrean, 2009; Ciocârlan, 2009; Sârbu et al., 2013). In Romania, *L. loeselii* is associated with *Schoenetum nigricantis* Oberd. 1957, corresponding to two distinct Romanian habitat types: R5413 - Southeastern Carpathian meso-

eutrophic bogs with *Carex davalliana* and R5414 - Southeastern Carpathian eu-mezotrophic fens with *Schoenus nigricans*. These habitats align with the Natura 2000 habitat type 7230 (Alkaline fens) (Figure 6).



Figure 6. The summer vegetation aspect of habitat 7230 (Alkaline fens) within the Hărman Natura 2000 site (photo by Roxana-Georgiana Nicoară, 09.06.2011)

In Ceahlău National Park, the last recorded observation dates back to 1989 (Mititelu), and in Piatra Craiului National Park, *L. loeselii* has not been confirmed despite multiple survey efforts, including those conducted under the POS Mediu project.

Similarly, in the Danube Delta, the species has not been reconfirmed since Kanitz (1879-1881), with Sârbu et al. (2006) failing to locate it in their surveys. Because the optimum conditions for its existence are alkaline fens (Doniță et al., 2005a; Doniță et al., 2005b; Ciocârlan, 2009) and this habitat is distributed only in intramountainous depressions (Gafta & Mountford, 2008; Goriup, 2008; INCDPM, 2014), we argue that it was an identification error for the existence of *L. loeselli* in Danube Delta.

Currently, *L. loeselii* is only confirmed in three Natura 2000 sites: ROSCI0074 Făgetul Clujului - Valea Morii, ROSCI0170 Pădurea și mlaștinile eutrofe de la Prejmer and ROSCI0055 Dealul Cetății Lempeș - Mlaștina Hărman with an estimated population size between 500 and 1000 individuals, although this estimate is based on extrapolated data.
A small population of *L. loeselii* may still persist in the Stupini Mire, which was proposed in 2024 as a new Natura 2000 site, highlighting its ecological importance and the potential need for enhanced protection and monitoring measures.

CONCLUSIONS

Liparis loeselii (L.) Rich., is critically endangered in Romania, with a highly fragmented distribution. Despite being present in 475 Natura 2000 sites across Europe, only three sites in Romania still harbor viable populations, emphasizing the urgent need for conservation and habitat restoration.
Historical records indicate significant population declines, with many previously cited locations failing to confirm the species' presence in recent surveys.
The conservation status is "unfavourable-inadequate" (U1), due to increasing trends in the intensity of different impacts upon the species habitat (Table 2).

Table 2. Overall assessment of the conservation status on each reporting period of *Liparis loeselii* (L.) Rich. in Romania

Species	2013		2019		2025	
	ALP	CON	ALP	CON	ALP	CON
Liparis loeselii (L.) Rich.	U1	U1	U1	U1	U1	U1

List of pressures and threats and conservation measures was updated in 2024 for the period 2019-2024 (EIONET, 2025).

Main conservation measures according with EU code: CA04 - Reinstate appropriate agricultural practices to address abandonment, including mowing, grazing, burning or equivalent measures. Conservation measures are taken inside or outside Natura 2000 sites where species is present.
According to standard Natura 2000 data forms, in Romania the species is listed in ten sites. The potential discrepancy between our results and the Natura 2000 data is the fact that the *L. loeselli* was initially listed in the standard data forms of the sites based on literature data, but subsequent field studies have failed to identify any populations. Due to the small size of the species, its conservation significance and the difficulty of locating individuals, there is a justified reluctance to remove the species from the data forms, therefore contributing to the inconsistency.

The study underscores the importance of long-term monitoring, habitat protection, and the implementation of conservation strategies to prevent further loss of this rare orchid.

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THE EFFECTIVENESS OF SOME BIOLOGICAL PRODUCTS USED TO CONTROL *CYDIA POMONELLA* L. POPULATIONS AND THEIR POTENTIAL FOR INTEGRATED PEST MANAGEMENT IN APPLE ORCHARDS

Cecilia BOLBOSE[1, 3], Maria IAMANDEI[2], Daniel Florin ȚONE[3], Cezarina NECULA[3, 4]

[1]Research Station for Fruit Growing Voinești, Principală Street, 137525, Voinești, Dâmbovița County, Romania
[2]Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, District 1, 013813, Bucharest, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Doctoral School, 59 Mărăști Blvd, District 1, Bucharest, Romania
[4]University Valahia of Târgoviște, 11 Sinaia Alley,Târgoviște, Dâmbovița County, Romania

Corresponding author email: maria_iamandei@yahoo.com

Abstract

The codling moth Cydia pomonella L. is a major pest of apple and other Rosaceae worldwide. Because in practice chemical control is intensively used to limit the damage of the codling moth, there is an urgent need for the development of sustainable control practices, involving non-chemical means. The aim of the study was to evaluate the efficacy of Bactospeine Df (T2), Laser 240 SC (T3) and Madex Top (T4) applied for C. pomonella control compared with chemical reference Coragen (T5) and untreated check (T1). The research was performed in the period 2023-2024, in an intensive apple orchard at R.S.F.G. Voinesti, with disease-resistant varieties Florina. In 2023 and 2024, the treatments efficacy at harvest ranged from 91.5% in treatment 4 to 95% in treatment 2 and 5 while the apple attack in untreated check was 24.25% and 33%. As a main conclusion, all the biological control products tested during the study have good potential for C. pomonella control and further inclusion in the integrated pest management in apple orchards from Dâmbovița fruit growing basin.

Key words*: Cydia pomonella, codling moth, biological control, apple orchards, integrated pest management.*

INTRODUCTION

A wide range of fruits, berries, and nuts are produced in Europe, where apple orchards represent the second most common single species and cover 0.5 million hectares.

From the estimated 35.4 million tons of fruits that were harvested in 2023, 12.0 million tons were apples. In 2023, in term of the surface of apple tree orchards, Romania, with 542900 hectares, comes on the 2nd position in the EU-28, after Poland, but was ranked only on 5th position on harvested production (EUROSTAT, 2024).

In recent years, the production of apple is often declined due to abiotic stresses associated with climate change, directly or indirectly, through the changes in the distributions and impacts of plant pests. Among the pest, over 50 species of Lepidoptera are known to attack the apple orchards (Rădulea et al., 2022). Those pest species that directly attack fruits are the biggest concern in apple production.

The codling moth, *Cydia pomonella*, is the most harmful insect species of the Tortricidae family that causes economic damage to apple production worldwide (Balasko et al., 2020). *C. pomonella* is a polyphagous species, and a major pest of apple and other pome fruits, and is found in almost all areas worldwide where apple trees are grown (Maggy & Chreil, 2023). The larvae bore into the fruit where they create tunnels, filled with frass, causing the fruit to rot or fall prematurely. The attacked fruits no longer develop normally, lose their commercial value and cannot be stored. If not properly managed, the fruits damage reaches up to 70% or more in unkempt orchards (Bolbose, 2022). Besides economic losses coming from direct yield reduction and post-harvest quality issues, producers are forced to invest more in crop protection. Codling moth control is the reason

for approximately 90% of insecticides' use in apple orchards in Provence, one of the main regions of apple production in France (Bosshardt et al., 2024).

In Romania, over an entire growing season, out of the 10-15 treatments applied in apple orchards, 6 to 8 are used for the codling moth control (Cârdei et al., 2007). The intense application of chemical insecticides is the usual option in great majority of apple orchards, where some populations of codling moth have developed resistance.

The alarming increase of the intensity of the codling moth attack in most plantations from Dâmbovița Fruit Growing Basin, has required reconsidering the annual phytosanitary treatment program and approaching an appropriate strategy that would reduce the number of pests in order to limit damage but also minimize the chemical impact on the environment and human health. The use of biological control agents (entomopathogens, predators and parasitoids) play key roles in IPM strategies (Lacey & Unruh, 2005), are intensively studied and several biological insecticides are available for codling moth control (Bolbose, 2022).

In this context, the aim of the study was to evaluate the efficacy of three biological control products as option for *C. pomonella* control and further inclusion in the integrated pest management program in apple orchards from Dâmbovița Fruit Growing Basin.

MATERIALS AND METHODS

Study was conducted over two years, 2023-2024, at farm no 1 of the Research Station for Fruit Growing Voinești (RSFG Voinești), in an intensive apple orchard, with disease-resistant variety Florina (45.086204, 25.235506), with the trees grafted onto the MM106 rootstock, loose palmette crown shape, and planting distances 4 x 2 m.

A randomized complete block design with four blocks and four replicate plot per treatment was used both years. Plot size was of 5 trees.

Four commercially available insecticides, three biologically and a chemical check, were used both years. The details about treatments are presented in Table 1.

All the other plant protection products (fungicides and herbicides) used, were applied uniformly to all plots, and comply with good standard practice.

Table 1. Treatment details

Treatment number/code	Specification	Active ingredient	Dose
T1	Untreated check	-	-
T2	Bactospeine DF	*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351	1 kg/ha
T3	Laser 240 SC	Spinosad	0.6 l/ha
T4	Madex Top	*Cydia pomonella* granulovirus (CpGV)	0.1 l/ha
T5	Coragen	Rynaxypyr®	0.15 l/ha

The calendar of application, presented in Table 2, was decided based on the flight periods of the codling moth adults, monitored with traps baited with AtraPOM pheromone lure (Figure 1), produced by "Raluca Ripan" Chemistry Institute in Cluj, Romania.

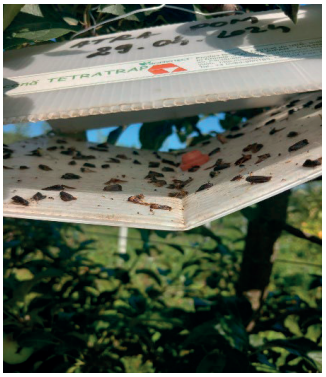


Figure 1. Pheromone trap used to monitor *Cydia pomonella* adult flight

There are usually two generations of codling moths per year. The first application to each generation was made at warning, respectively when 5 *Cydia pomonella* adults were recorded per week. Thereafter, according to label specifications of every plant protection product, there were made 0 to 2 applications, per each generation at an interval of 7-8 days.

Table 2. Callendar of applications in 2023 and 2024

	1st generation			2nd generation			
Year	Warning	A1	A2	Warning	A1	A2	A3
2023	16.05.	17.5	23.5	18.7	19.7	28.7	-
2024	13.05	14.5	22.5	14.07	15.7	23.7	2.8

Note A1- Application 1

Assessment of attacked fruits were made at three moments each year: (i) before treatment application, (ii) at the end of the first codling moth generation (28th June 2023 and 30th June 2024) and at harvest (17th September 2023 and 27th September 2024). At each assessment, three hundred fruits per plot, present on trees or on the ground, were visually examined, the number of fruits attacked by *C. pomonella* larvae were counted. Statistical data processing was carried out by Student (t) test for the confidence interval of 95% and Abott formula for treatment efficacy.

RESULTS AND DISCUSSIONS

The experimental conditions were favourable to pest dynamic, the first generation of codling moth was recorded from the first decade of May (10.05.2023 and 7.05.2024) and lasted until mid-June (18.06.2023, 12.06.2024). The first catches of second generation were recorded at 11.07.2023 and 7.07.2024 and lasted till 21.08.2023 and 3.09.2024.

The fruit damage gradually increased in untreated control plots from 0% at the beginning of observations to 24.25 % at harvest in 2023 respectively 33% in 2024.

The results obtained from testing the biological products, compared to the standard chemical product and the untreated reference are presented in the Tables 3 and 4.

In 2023, at the end of the first generation of the pest, the average frequency of attack was between 5.5% in case of treatment 5 (chemical reference Coragen), followed by treatment 2 (Bactospeine DF) with 6%, both were found statistically at pair and significant superior comparing to T3 (Laser 240 SC, 8.5%) and T4 (Madex Top, 9.5%) and very significant superior to the T1 untreated plots where the average frequency was of 16.5%. The situation was similar at harvest, the minimum average frequency of attack was similar in case of T5 and T2 (5.25%), followed by T3 (7.25%) and T4 (9.25%).

The percent of control (saved fruit on tree per treatment) was between 91.5 in treatment 4 and 94.75 in case of T2 and T5.

In 2024, at the end of the first generation of the pest, the average frequency of attack was between 5% in case of treatment 5 (chemical reference Coragen), followed by treatment 2 (Bactospeine DF) with 6.5%, both were found statistically at pair and significant superior comparing to T4 (Madex Top, 7.5%), followed by T3 (Laser 240 SC, 8%) and and very significant superior to the T1 untreated plots where the average frequency was of 24.5%. At harvest, the best efficacy results were in T5 and T2 (95%), followed by T3 (94%), and T4 (93.5%). All of them were significantly superior comparing to untreated check.

Table 3. Efficacy of various treatment applied for *C. pomonella* control in 2023

Treatment number/ code	Specification	Active ingredient	Dose	No of applications	% of attacked fruit			Pest control %
					Before A1	At the end of 1st generation	At harvest	At harvest
T1	Untreated check	-	-	-	0.00	16.5 a	24.25 a	-
T2	Bactospeine DF	*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351	1 kg/ha	4	0.00	6.00 d	5.25 d	94.75
T3	Laser 240 SC	Spinosad	0.6 l/ha	4	0.00	8.5 c	7.25 c	92.75
T4	Madex Top	*Cydia pomonella* granulovirus (CpGV)	0.1 l/ha	3	0.00	9.5 b	9.25 b	91.5
T5	Coragen	Rynaxypyr®	0.15 l/ha	2	0.00	5.5 d	5.25 d	94.75

Table 4. Efficacy of various treatment applied for *C. pomonella* control in 2024

Treatment number/code	Specification	Active ingredient	Dose	No of applications	% of attacked fruit			Pest control %
					Before A1	At the end of 1st generation	At harvest	At harvest
T1	Untreated check	-	-	-	0.00	24.75 a	33.0 a	-
T2	Bactospeine DF	*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351	1 kg/ha	5	0.00	6.50 c	6.25 c	95.0
T3	Laser 240 SC	Spinosad	0.6 l/ha	4	0.00	8.0 b	7.25 bc	94.0
T4	Madex Top	*Cydia pomonella* granulovirus (CpGV)	0.1 l/ha	3	0.00	7.5 b	7.75 b	93. 5
T5	Coragen	Rynaxypyr®	0.15 l/ha	2	0.00	5.0 d	6.25 c	95.0

The results are in line with previous data from the same area (Bolbose, 2022) that showed good control of *C. pomonella* in case of treatment conducted with Madex Top (94.34-95.00% fruit free from attack), and Laser 240 SC (92.67-94.00%).

CONCLUSIONS

Under the conditions of Voinești fruit growing area, during the years 2023-2024, the development of 2 generations of codling moth was recorded, starting from the first decade of May (1st generation) and second decade of July (2nd generation). In 2024, the flight period of the second generation of codling moth was longer, with the last catches recorded in September and fruit damage increased compared with previous year.

All the three biological products significantly controlled codling moth infestation compared with untreated control.

Overall, the product Bactospeine DF showed a verry good efficacy, very closed to those of the chemical reference Coragen.

Results of this experiment will be helpful in further development of Integrated control program of codling moth for Dâmbovița fruit growing basin and will also be very helpful to local apple growers for selection of effective insecticide for the management of *Cydia pomonella* populations.

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MINERAL COMPOSITION OF WILD CRANBERRIES HARVESTED FROM APUSENI MOUNTAINS, ROMANIA

Gabriela BRAD[1, 3], Adrian PETICILĂ[1, 3], Corina PREDESCU[1, 5], Nicoleta HĂDĂRUGĂ[2, 4], Liliana TUDOREANU[1, 5]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului, Timișoara, Romania
[3]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[4]Faculty of Food Engineering, University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului, Timișoara, Romania
[5]Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independenței, District 5, Bucharest, Romania

Corresponding author email: gabriela.brad@doctorat.usamv.ro

Abstract

Wild cranberries represent a constant source of income for many local communities, however during the last decade, a few studies were published concerning their nutritional quality. The aim of the present study is to identify the influence of harvest time and location on mineral concentrations of wild cranberry berries from Apuseni Mountains, Mărgău County, Romania. Fifteen cranberry samples from Apuseni Mountains from 3 different locations were collected in August and September 2024. Minerals such as Ca, Mg, K, Zn Fe, Cu, B, Sr, were analyzed in all samples by ICP-OES. The highest Calcium concentration (177.40 ± 1.27 mg/kg) was identified for Vlădeasa-Răzoare location (September harvest). No significant differences (P>0.05) were identified for Ca, and K concentrations (regardless of the harvest time) and no significant difference for Zn and Fe total concentrations (August harvest) for berries from Vlădeasa-Răzoare and Vlădeasa-High locations. The characterization of the mineral profile of wild cranberry from Apuseni Mountains, Mărgau County, is facilitating the identification of the best harvest time and location for the development of local produces with protected designation of origin.

Key words*: cranberry, minerals, harvest time.*

INTRODUCTION

Cranberry (*Vaccinium vitis-idaea* L.) is a perennial species from the Ericaceae family, recognized for its rich content of bioactive compounds, including vitamins, polyphenols, and essential minerals. These fruits are valued both for their nutritional benefits and their traditional and modern applications in phytotherapy. The mineral composition of wild cranberries is influenced by various factors, including edaphic and climatic conditions. Little information on mineral composition of wild cranberries was published, therefore there is a high need for detailed studies on the variability of minerals concentrations depending on the region of origin (Cristea, 2023; Vollmannová, 2018; Karlsons, 2018; Shotyk, 2020).

The Apuseni Mountains represent an ideal natural habitat for the spontaneous flora of cranberries due to their acidic soils and specific climatic conditions. Comparisons between Vaccinium species harvested from spontaneous flora and those from controlled crops have revealed significant differences in the content of macro- and microelements, particularly for elements such as calcium, magnesium, iron, and zinc (Karlsons, 2018; Shotyk, 2019; Vollmannová, 2019; Shotyk, 2020). Moreover, it has been demonstrated that cranberries from unpolluted areas, such as peat bogs and isolated mountain regions, exhibit a distinct mineral profile compared to those from areas affected by anthropogenic activities (Shotyk, 2019; Vollmannová, 2018; Tomáš, 2019; Shotyk, 2020).

Previous studies have shown that fruits of the Vaccinium genus, including cranberries, are influenced by the composition of the substrate on which they grow and the availability of nutrients in the soil (Karlsons, 2022; Vollmannová, 2018; AgricultForest, 2023; Shotyk, 2020). For example, research on cultivated cranberries has demonstrated that nitrogen and phosphorus fertilization can significantly modify the mineral content of the fruits (Karlsons, 2022; Vollmannová, 2018). Additionally, isotopic analyses and chemometric studies have been used in differentiating wild berries from cultivated ones, providing essential information regarding the authenticity and traceability of cranberry products (Cristea, 2023; Karlsons, 2018; Shotyk, 2020).

The mineral content analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES), a method recognized for its precision and sensitivity in determining chemical elements in plant matrices (Vollmannová, 2018; Shotyk, 2019; Tomáš, 2019; AgricultForest, 2023; Shotyk, 2020).

In this context, the aim of the present study is to analyze the mineral content of spontaneously growing cranberries harvested from the Apuseni Mountains, highlighting potential differences compared to other regions and identifying the factors contributing to this variability. The study aims to provide relevant data for assessing the quality of these fruits and contribute to the scientific literature on the impact of environmental factors on the mineral composition of wild plants.

MATERIALS AND METHODS

Samples collection and preparation

In 2024 fifteen wild cranberry samples were harvested from three different locations in Apuseni Mountains, Mărgău County, Romania, from August to September. For each location, 3 samples formed of 5 g of fresh wild cranberries were used to determine the mineral composition. The samples were prepared based on a modified method of Dróżdż et al. (2018) using 5 grams of fresh cranberries. Fruits were grinded and homogenised, using GrindomixGM200, Retsch GmbH, Haan, Germany.

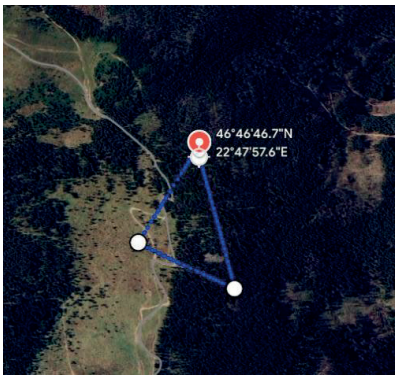


Figure 1. Harvest area in Apuseni Mountains, Romania

For each location 2 kg of fruits were harvested: from location Boaică only one harvest was made on 14 August 2024. Due to extreme drought, no second harvest was possible on Boiacă location. Two harvests were made for locations Vlădesa-Răzoare, VR_1 and VR_2, and Vlădeasa-Sus, VS_1 and VS_2, on 16 August and 7 September 2024 respectively. From each harvest, three samples were extracted and used for mineral analysis.

Macroelements and microelements analysis

Elements Ag, Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, Tl, Zn were analysed by ICP OES Thermo Scientific model iCAP 6000. Only the macroelements Ca, K, Mg, Na and microelements Al, Ba, Cu, Fe, Mn, Sr, Zn concentrations exeeeded the detection limit of the method. The operating parameters of the spectrometer were: Power 1.15 kW, auxiliary Ar gas flow rate 0.2 L/min, nebulizing Ar gas flow rate 0.42 L/min, coolant gas flow rate of 12 L/min. The numberd of redings per sample: 3. The lines selected were: Al 308.2 nm, Ba 233.5 nm; Ca 422.6 nm, Cu 224.7 nm; Fe 255.9 nm; K 766.4 nm; Mg 279.5 nm; Mn 257.6 nm; Na 589.5 nm; Sr 407.7 nm; Zn 206.2 nm. For the others elements, all lines selected showed that the concentrations are below the detection limit of the method.

Statistical analysis

The statistical analysis was performed using MedCalc® Statistical Software version 22.018 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024).

Comparisons were made for each mineral between locations regardless the harvest time. Data normality was analyzed using Shapiro-Wilk test, equality of error variances was analyzed using Levene's test. When data were normally distributed and ANOVA was significant ($p<0.05$) a post hoc analysis was made using the Tuckey-Kramer test. We found that all data were normally distributed. For samples having unequal variances of elements' concentrations data, the Welch-ANOVA test allowing unequal variance was applied. To compare the data generated by our research with the published data we used one sample t test using the MedCalc software.

RESTULTS AND DISCUSSIONS

Macroelements concentration in wild cramberries fuits from Apuseni mountains

The maximum Ca total concentrations (177.4011 mg/kg fresh weight) was recorded for cranberries harvested in the VR area at the end of the harvesting period (Table 1, Figure 2). The minimum Ca concentration (149.1665 mg/kg fresh weight) was found for the fruits harvested from Boaică area at the beginning of the harvesting period (Table 1).

Table 1. Macroelements concentrations (mg/kg fresh weight) in wild cranberries harvested from Apuseni Mountains, Romania. Data can be compared only between harvests for the same element. Values followed by the same letter are not significantly different (p=0.05)

Harvest	Ca	K	Mg	Na
VR_1	$152.65^{a}\pm 3.63$	$648.96^{b}\pm 42.47$	$62.38^{b}\pm 3.46$	$11.76^{ab}\pm 0.89$
VR_2	$177.40^{b}\pm 1.27$	$811.88^{a}\pm 6.59$	$74.78^{a}\pm 1.29$	$10.75^{ab}\pm 1.46$
VS_1	$155.13^{ab}\pm 10.11$	$645.95^{b}\pm 19.77$	$62.98^{b}\pm 3.03$	$12.10^{ab}\pm 1.91$
VS_2	$165.92^{ab}\pm 15.61$	$732.18^{ab}\pm 38.49$	$66.89^{ab}\pm 6.70$	$12.92^{b}\pm 0.56$
BOAICĂ	$149.16^{a}\pm 5.98$	$750.28^{a}\pm 19.27$	$64.00^{ab}\pm 4.27$	$8.95^{a}\pm 0.54$

Ca concentration had a significant variation depending on both the harvest area and the harvest period. Specifically, the Ca concentration in samples collected from the Boaica area at the beginning of the harvest period are significantly lower from those collected in the Vlădeasa-Răzoare (VR_2) area at the end of the harvesting period (Table 1, Figure 2). Additionally, Ca concentrtions in fruits from VR area at the beginning of the harvesting period differ significantly of Ca concentrations from fruits collected in the same area at the end of the harvesting period.

The K (potassium) maximum concentration was measured from the fruits harvested in VR area at the end of the harvesting period (811.8808 mg/kg fresh weight). The minimum K concentration (645.9591 mg/kg fresh weight) was found for the cranberries harvested in the VS area at the beginning of the harvesting period (Table 1, Figure 2). The K concentrtions in cranberries fruits collected from the Boaica area at the beginning of the harvesting period were significantly different from K concentrations in cranberries fruits collected in the VS and VR areas at the beginning of the harvesting period (Table 1, Figure 2).

Additionally, K concentrations for fuits from the VR area at the beginning of the harvesting period differ significantly from the K concentrations of the fruits collected in the same area at the end of the harvesting period. Furthermore, K concentrations in cranberries from VS area at the beginning of the harvesting period differ significantly from those collected in the VR area at the end of the harvesting period. Therefore. K concentration varies depending on both the harvesting area and the harvesting period. For VS and VR location there was no significat difference between K concentrations in cranberries harvested in the same day (Table 1, Figure 2).

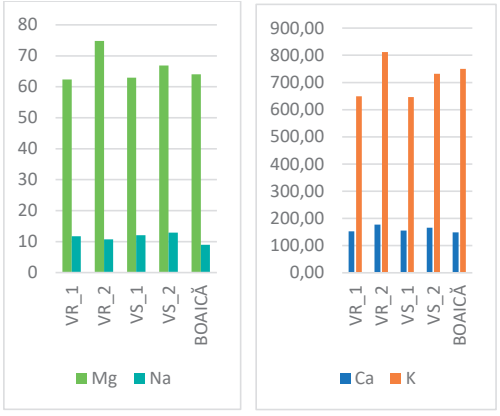


Figure 2. Variation of Ca, K , Mg and Na concentrations by location and harvest time

For Mg, the maximum concentrations is 74.7819 mg/kg fresh weight for VR_2 area and the minimum concentration was found to be 62.9848 mg/kg fresh weight for VS_1 (Table 1, Figure 2). The Mg total concentrations in cranberry fruits collected from the VR area at the beginning of the harvesting period differ significantly from those collected in the same area at the end of the harvesting period (Table 1, Figure 2). Therefore, Mg concentration varies depending on the harvesting time.

For Na, the maximum value recorded for concentration is 12.9299 mg/kg in the VS area at the end of the harvesting period (Table 1, Figure 2). The minimum Na concentration is 8.9581 mg/kg in the Boaica area at the beginning of the harvesting period. The Na concentration in fruits collected from the Boaica area at the beginning of the harvesting period differ significantly from those collected in the VS area at the end of the harvesting period (Table 1, Figure 2). Magnesium and potassium are conssidered to have a high mobility in the phloem, which may explain the significant accumulation of Mg and K during the cranberries fruits development and ripening for the VR and VS locations.

Comparing the data from Ca, K, Mg concentrations of wild cranberries harvested in the Apuseni Mountains with data reported by Karlson et al. (2018) for cultivated varieties of cranberries in Latvia showed that there is a significant difference between cultivated and wild fruits mineral concentrations (Table 2), wild fruits from Apuseni mountains being reacher in macronutrients.

In Table 2 the minerals that were not signifficantly different from the publisehed literature (Karlsons et al., 2018) are in italics and in higlighted cells. The Mg concentrations for the V. Macrocarpon cultivar is mostly similar to the Mg concentrations in wild cranberries from Apuseni mountains except for the VR_2 harvest. Due to the fact that Karlsons et al. (2018) did not reported Na concentrations, it was not possible to make a comparison between the Apuseni mountains' wild cranberries fruits Na total concentations and the published data for cultivated cranberries. The Ca concentration values in cranberry fruits collected from the Apuseni Mountains differ significantly from the Ca concentration values reported by Karlsons et al. (2018). Thus, the Ca concentration recorded in VR_1 is 1.20 times higher than the Ca concentration in *V. oxycoccos* (p=0.0068) and 1.50 times higher than in *V. macrocarpon*, as reported by Karlsons et al. (2018).

Table 2. P values for the comparison (one sample t test) between macroelements concentrations (mg/kg fresh weight) in wild cranberries fruits harvested from Apuseni Mountains, Romania and the macroelements concentrations (mg/kg fresh weight) in American cranberry fruits (*Vaccinium macrocarpon*) and European cranberry (*Vaccinium oxycoccus*) fruits cultivated in Latvia (Karlsons et al., 2018)

Element	Location-wiled cramberries	*V. oxycoccos*	*V. macrocarpon*
Ca	VR_1	P=0.0068	P = 0.0017
	VR_2	P = 0.0018	P = 0.0008
	VS_1	P = 0.0416	P = 0.0118
	VS_2	*P = 0.0506*	P = 0.0192
	BOAICĂ	P = 0.0243	P = 0.0053
K	VR_1	*P = 0.4483*	*P = 0.0899*
	VR_2	P = 0.0007	P = 0.0019
	VS_1	*P = 0.1509*	P = 0.0202
	VS_2	*P = 0.1133*	*P = 0.7802*
	BOAICĂ	P = 0.0196	*P = 0.1519*
Mg	VR_1	P = 0.0128	*P = 0.3064*
	VR_2	P = 0.0145	P = 0.0073
	VS_1	P = 0.0094	*P = 0.2164*
	VS_2	*P = 0.0685*	*P = 0.8571*
	BOAICĂ	P = 0.0206	*P = 0.4840*

For K, the values recorded in VR_2 and Boaică differ significantly from the K concentration values reported by Karlsons et al. (2018) for *V. oxycoccos* and *V. macrocarpon*. Specifically, the K value recorded in VR_2 is 1.21 times higher than the value reported by Karlsons et al. (2018) for *V. oxycoccos* (p=0.0007) and 1.12 times higher than the value reported for *V. macrocarpon* (p=0.0019).

For Mg, the values recorded in VR_1, VR_2, VS_1, and Boaică differ significantly from the values reported by Karlsons et al. (2018) for *V. oxycoccos* and *V. macrocarpon*. Thus, the Mg concentration recorded in VR_1 is 0.77 times

lower than the value reported by Karlsons et al. (2018) for *V. oxycoccos* (p=0.0128).

Oligoelements concentrations in wild cranberries fruits from Apuseni region.

Microelements such as Cu, Fe, and Mn are associated with plant enzyme systems, therefore, it is expected that their concentration increase in berries over the harvest period. Al does not play a phisiological role.

The maximum Al total concentration was found in VR at the beginning of the harvesting period (15.9519 mg/kg fresh weight) (Table 3, Figure2). The minimum Al total concentration was 4.3644 mg/kg in the Boaica area at the beginning of the harvesting period (Table 3, Figure 3).

Table 3. Oligoelements concentration(mg/kg) in wild cranberries harvested from Apuseni Mountains. Romania. Data can be compared only between harvests for the same element. Values followed by the same letter are not significantly different (p=0.05)

Harvest	Al	Ba	Cu	Fe
VR_1	$15.95^{b}\pm 5.15$	$1.30^{a}\pm 0.04$	$1.03^{a}\pm 0.11$	$3.04^{b}\pm 0.16$
VR_2	$7.88^{a}\pm 3.62$	$1.97^{b}\pm 0.08$	$1.19^{ab}\pm 0.07$	$3.38^{b}\pm 0.52$
VS_1	$9.48^{ab}\pm 0.86$	$2.07^{b}\pm 0.05$	$1.27^{b}\pm 0.04$	$3.96^{b}\pm 0.31$
VS_2	$8.90^{ab}\pm 1.49$	$2.19^{bc}\pm 0.24$	$1.24^{ab}\pm 0.05$	$7.00^{a}\pm 1.37$
BOAICĂ	$4.36^{a}\pm 0.09$	$1.72^{bd}\pm 0.12$	$1.23^{ab}\pm 0.01$	$2.59^{b}\pm 0.05$

Table 4. Oligoelements concentration in wild cranberries harvested from Apuseni Mountains. Romania (mg/kg). Data can be compared only between harvests for the same element. Values followed by the same letter are not significantly different (p=0.05)

Location	Mn	Sr	Zn
VR_1	$15.16^{ab}\pm 1.38$	$0.23^{a}\pm 0.007$	$15.23^{b}\pm 0.50$
VR_2	$20.37^{b}\pm 2.73$	$0.30^{a}\pm 0.01$	$4.85^{a}\pm 3.86$
VS_1	$17.88^{b}\pm 1.48$	$0.49^{b}\pm 0.009$	$16.03^{b}\pm 1.12$
VS_2	$15.62^{ab}\pm 2.25$	$0.48^{b}\pm 0.06$	$15.25^{b}\pm 0.16$
BOAICĂ	$11.82^{a}\pm 1.62$	$0.23^{a}\pm 0.04$	$2.18^{a}\pm 0.22$

For cranberries from the VS area, there are no significant differences in Al concentration regardless of the harvesting period. This is probably due to a poor soil Al concentrations in the VS region. Al mobility in soild is higher for acidic and neutral pH soil but lower for alkaline soil, and its mobility is reduced by soil organica matter (Zeiner and Cindric; 2018). We also suspect that VS soils have a higher organic mater and thus reduce aluminium availability for plants. The presence of Al and Fe in the soils of this region has a natural occurance, due to the fact that VS, VR an Boaică areas are situated in metalliferous mountains, naturally rich in non-ferrous and ferrous minerals. For cranberries from the VR area there is a significant difference in Al concentrations depending on the harvesting period.

Zinc is involved in the synthesis of tryptophan (precursor of auxin) therefore it helps in reducing fruit drop. The significant dicrease in fruit Zn For the VR_2 compared to VR_1 might be explained by a redused soil bioavailability during the second harvest period. Soil Zn bioavailability is influenced by organic matter and pH.

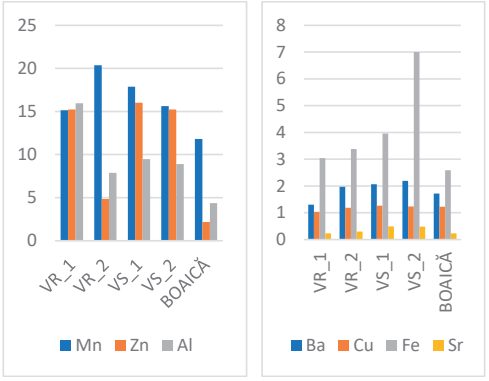


Figure 3. Variation of Al, Ba, Cu Fe, Mn, Sr and Zn concentrations by location and harvest time.

Ba maximum concentration (2.1989 mg/kg fresh weight) was found in berries from VS area at the end of the harvesting period (Table 2, Figure 3). The Ba minimum concentration (1.3031 mg/kg fresh weight) was found for the berries harvested from VR area at the beginning of the harvesting period. However, the berries harvested in VS area did not show significant differences of Ba concentration regardless of the harvesting period. In contrast, VR berries showed significant differences in Ba concentration depending on the harvesting period. Barium is a silvery-white metal which is naturalrely found in soils in combination with chemicals, such as sulfate, carbonate, and

hydroxide. Barium may be present in air as a result of antropogenic emissions, in particular by the burning of coal and diesel. Local population of the research area is using coal as well as wood for heating and for daily household needs. As a consequence due to its natural presence in the soil and antropogenic emission, it can enter the food chain through root and foliar uptake by plants. Plants are known to be able to uptake Ba and acumulate in storage organs as well as in leaves, therefore foods such as lettuce, onions, beans, potatoes, nuts, and cereal grains may contain Ba (Lee et al., 2023)

The maximum Cu concentration was recorded in the VS area at the beginning of the harvesting period, reaching 1.2779 mg/kg fresh weight (Table 3, Figure 3). The minimum value was recorded in the VR area at the beginning of the harvesting period (1.0380 mg/kg fresh weight) (Table 3, Figure 3). The Cu concentration does not differ significantly based on the harvesting period, however, it varies significantly depending on the harvesting area. Specifically, the samples from the VR area differ significantly from those in the VS area at the beginning of the harvesting period.

While calcium and manganese have a low phloem mobility, elements such as copper. iron and zinc have a variable or conditional phloem mobility depending on plant species, environmental influences, plant tissue and growth stage. For VR and VS locations there is an evident increase of Fe concentration during the ripening period. For Fe, the maximum value was recorded in the VS area at the end of the harvesting period, reaching 7.0075 mg/kg fresh weight (Table 3, Figure 3). The minimum value was recorded in the Boaica area at the beginning of the harvesting period, at 2.5966 mg/kg fresh weight (Table 3, Figure 3). Significant differences in Fe concentration were observed based on both the harvesting area and the harvesting period. A possible explanation for this situation is the existence of a higher moisture and raifall in the VS region compared to the other regions which were drastically affected by drought. Despite copper and iron are conssidered to have a variable or conditional phloem mobility, their accumulation continued throughout the cranberry fruits development and ripening. Therfore their accumulation into the fruits might conssidered to be through the phloem.

For Mn, the maximum recorded value was 20.3713 mg/kg fresh weight in the VR area at the end of the harvesting period (Table 4, Figure 3). The minimum value was recorded in the Boaică area at the beginning of the harvesting period, at 11.8236 mg/kg fresh weight (Table 4, Figure 3).

Regarding Fe concentration, the cranberry fruits collected from the Boaica area differ significantly from those collected in the VR area at the end of the harvesting period, as well as from those collected in the Vlădeasa Sus area at the beginning of the harvesting period (Table 4, Figure 3). Therefore, Fe concentration differs significantly depending on both the harvesting area and the harvesting period.

For Sr, the maximum recorded value is 0.4948 mg/kg fresh weight in the VS area at the beginning of the harvesting period (Table 4, Figure 3). The minimum value was recorded in the Boaică area, at 0.2305 mg/kg fresh weight, also at the beginning of the harvesting period (Table 4, Figure 3). The Sr concentration differs significantly depending on the harvesting area, with cranberry fruits from the Boaică and VR areas differing significantly from those in the VS area.

The maximum Zn concentration was recorded in the VS area at the beginning of the harvesting period, reaching 16.0337 mg/kg fresh weight (Table 4, Figure 3). The minimum value was recorded in the Boaică area, also at the beginning of the harvesting period, at 2.1806 mg/kg fresh weight (Table 4, Figure 3). Zn concentration values differ significantly depending on both the harvesting area and the harvesting period. Specifically, the fruits collected from the Boaica area at the beginning of the harvesting period and those from the VR area at the end of the harvesting period differ significantly from the fruits collected from the VR area at the end of the harvesting period, as well as from those collected in the VS area during both harvesting periods (Table 4, Figure 3).

In Table 5 the minerals that were not signifficantly different from the publisehed literature (Karlsons et al.,2018) are in italics and in higlighted cells. Due to the fact that Karlsons et al.(2018) did not reported Al, Ba and Sr concentrations, it was not possible to make a comparison between the Apuseni mountains'

wild cranberries fruits Al, Ba and Sr total concentations and the published data for cultivated cranberries. The Cu concentration values in cranberry fruits collected from the Apuseni Mountains differ significantly from the Cu concentration values reported by Karlsons et al. (2018) for both *V. oxycoccos* and *V. macrocarpon*. Thus, the Cu concentration in fruits collected from VR_1 is 1.72 times higher than the value reported by Karlsons et al. (2018) for *V. oxycoccos* (P = 0.0211) and 2.10 times higher than the Cu concentration reported for *V. macrocarpon* (P = 0.0136).

Table 5. P values for the comparison (one sample t test) between oligoelements concentrations (mg/kg fresh weight) in wild cranberries fruits harvested from Apuseni Mountains, Romania and the oligoelements concentrations (mg/kg fresh weight) in American cranberry fruits (*Vaccinium macrocarpon*) and European cranberry (*Vaccinium oxycoccus*) fruits cultivated in Latvia (Karlsons et al., 2018)

Element	Location-wild cranberries	*V. oxycoccos*	*V. macrocarpon*
Cu	VR_1	P = 0.0211	P = 0.0136
	VR_2	P = 0.0047	P = 0.0033
	VS_1	P = 0.0012	P = 0.0009
	VS_2	P = 0.0020	P = 0.0015
	BOAICĂ	P = 0.0001	P = 0.0001
Fe	VR_1	*P = 0.5826*	P = 0.0005
	VR_2	*P = 0.4495*	P = 0.0061
	VS_1	P = 0.0407	P = 0.0030
	VS_2	P = 0.0388	*P = 0.8240*
	BOAICĂ	P = 0.0032	P < 0.0001
Mn	VR_1	P = 0.0055	P = 0.0036
	VR_2	*P = 0.0725*	P = 0.0072
	VS_1	P = 0.0112	P = 0.0028
	VS_2	P = 0.0156	P = 0.0088
	BOAICĂ	P = 0.0044	P = 0.0088
Zn	VR_1	P = 0.0004	P = 0.0004
	VR_2	*P = 0.2717*	*P = 0.2821*
	VS_1	P = 0.0020	P = 0.0020
	VS_2	P < 0.0001	P < 0.0001
	BOAICĂ	P = 0.0332	P = 0.0448

The Fe concentration values in cranberry fruits collected from VS_1, VS_2, and Boaică differ significantly from the Fe concentration values reported by Karlsons et al. (2018) for *V. oxycoccos*. Specifically, the Fe concentration in fruits from VS_2 is 2.26 times higher than the Fe concentration reported by Karlsons et al. (2018) for *V. oxycoccos* (P = 0.0388).

The Fe concentration values in fruits collected from VR_1, VR_2, VS_1, and Boaică differ significantly from the Fe concentration values reported by Karlsons et al. (2018) for V. macrocarpon. For instance, the Fe concentration in cranberries collected from Boaică is 0.36 times lower than the value reported by Karlsons et al. (2018) for V. macrocarpon (P < 0.0001).

The Mn concentration values in fruits collected from VR_1, VS_1, VS_2, and Boaică differ significantly from the Mn concentration values reported by Karlsons et al. (2018) for *V. oxycoccos*. Specifically, the Mn concentration in fruits collected from VR_1 is 0.59 times lower than the Mn concentration reported by Karlsons et al. (2018) for *V. oxycoccos* (P = 0.0055).

The Zn concentration values in cranberry fruits collected from VR_1, VS_1, VS_2, and Boaică differ significantly from the Zn concentration values reported by Karlsons et al. (2018) for both *V. oxycoccos* and *V. macrocarpon*. Thus, the Zn concentration in fruits collected from VR_1 is 10.15 times higher than the value reported by Karlsons et al. (2018) for *V. oxycoccos* and 9.25 times higher than the Zn concentration reported for *V. macrocarpon* (P = 0.0004).

CONCLUSIONS

The highest concentration of Fe was recorded in the samples collected from the Vlădeasa Sus area at the end of the harvesting period being significantly different from the samples collected from the other regions. The Mg, K and Ca total concentrations are significantly higher for the September harvest. The Na total concentrations were not significantly different over the whole harvest period. However, these results have to be considered with caution due to the large variability of the measurements. The Zn and Al total concentrations are decreasing during the harvest period, the cranberry fruits from the Boaică area contains the lowest concentrations of Zn and Al compared to the cranberry fruits harvested from the other Apuseni Mountains studied regions. The Fe total concentrations are significantly higher in the cranberry fruits harvested in September.

However, the Cu total concentrations are not significant.

Compared with the published total concentrations of Ca, K, Mg, Cu, Fe, Mn and Zn from *V. macrocarpon* cranberry cultivar and *V. oxycoccos* the cranberry variety from the Apuseni Mountains is reacher in minerals with few exceptions.

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RESEARCH ON THE UTILIZATION OF NUTRIENTS AND BIOACTIVE COMPOUNDS FROM CAULIFLOWER STALKS, THROUGH THE DEVELOPMENT OF A FUNCTIONAL INGREDIENT WITH HIGH NUTRITIONAL VALUE AND ANTIOXIDANT CAPACITY

Monica CATANĂ[1], Luminița CATANĂ[1], Adrian ASĂNICĂ[2], Monica-Alexandra DÂRĂ[1], Anda-Grațiela BURNETE[1], Florica CONSTANTINESCU[1]

[1]National Research and Development Institute for Food Bioresources, IBA Bucharest, 6 DinuVintilă, District 2, 021102, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: adrian.asanica@horticultura-bucuresti.ro

Abstract

Agricultural production and the food industry generate a large amount of waste, with a major impact on the environment. The Action Plan for the European Strategy for the Circular Economy includes a zero-waste strategy targeting agri-food waste to reduce environmental pollution. The aim of this study was to valorize cauliflower stalks in order to obtain a functional ingredient rich in nutrients and bioactive compounds, which confer antioxidant capacity. This functional ingredient appears as a powder with characteristic taste and smell, in a whitish-yellow or whitish-gray color, with a high content of proteins (23.12-24.09%), available carbohydrates (29.21-31.81%), total fiber (23.27-26.85%), mineral elements (K: 1785.35-2175.23 mg/100 g; Ca: 505.35-598.86 mg/100 g; Mg: 97.56-128.56 mg/100 g; Fe: 28.43-43.57 mg/100 g; Zn: 4.27-5.62 mg/100 g), bioactive compounds (total polyphenols: 4.52-5.47 mg GAE/g d.w.; glucosinolates: 7.28-8.20 µmol/g d.w.; vitamin C: 45.03-48.12 mg/100 g). The functional ingredient obtained from cauliflower stalks stands out for its ability to neutralize free radicals (10.72-12.94 µmol Trolox Equivalents/g).

Key words*: antioxidant capacity, cauliflower, functional ingredient, stalks.*

INTRODUCTION

According to the "Food 2030 Pathways for Action" (Directorate-General for Research and Innovation, 2020), primary production (25%) and processing (24%) account for approximately 50% of total food losses, with fruits and vegetables representing the largest share of these losses. In the early stages of processing, these losses are mainly caused by disposal due to high commercial standards, as well as edible parts that are removed during peeling or cutting in fresh processing lines (outer leaves, stalks, damaged parts).

It is necessary for production systems to focus on minimizing waste and reintroducing it into the production chain. In this regard, concepts such as "circular economy," "industrial ecology," and "zero-waste economy," among others, have emerged to guide eco-innovation towards using waste as raw material for the development of new products and applications (Ramírez-Pulido et al., 2021). Reducing food waste is also an important contribution to the Circular Economy Action Plan adopted by the European Commission within the European Green Deal, which promotes sustainable initiatives throughout the entire product lifecycle. A circular approach brings benefits to sustainable food production (European Commission, 2019).

The vegetable processing industry produces over one million tons of plant by-products, considered waste, every year, which represents a potential source of bioactive ingredients (Galali et al., 2020; Rafiuddin et al., 2019; 2021). International studies have shown that there is a huge potential in utilizing plant-based by-products for the development of food products (Amoah et al., 2023; San José et al., 2018; Tamasi et al., 2019). In this regard, cauliflower by-products (which account for about 60% of the cauliflower crop) are among the most important, distinguished by their content of nutrients and bioactive compounds (polyphenols, glucosinolates, carotenoids,

vitamin C, chlorophyll a, chlorophyll b, etc.). Thus, Drabińska et al. (2021) mentioned that the powder obtained from cauliflower stems stands out due to its ash content (11.78%), protein content (19.12%), and carbohydrates (64.36%). Additionally, based on their research, these authors highlighted that the powder from cauliflower stems is remarkable for its total amino acid content: 2422.64 µmol/g dry matter. The amino acids that are most abundant in this powder are as follows: Glutamine (1009.42 µmol/g dry matter), Valine (100.01 µmol/g dry matter), Alanine (341.32 µmol/g dry matter), Serine (268.31 µmol/g dry matter), Asparagine (205.97 µmol/g dry matter), Glutamic acid (193.56 µmol/g dry matter).

Furthermore, Drabińska et al. (2021) showed that the powder from cauliflower stems is rich in glucosinolates (125.05 mmol/g dry matter), total polyphenols (2.65 mg GAE/g dry matter), and chlorophyll b (0.66 µg/g dry matter). Tukassar et al. (2023) mentioned that cauliflower by-products powder is notable for its ash content (5.81%), protein content (22.80%), carbohydrates (47.62%), minerals (Mg = 363 mg/kg; Fe = 200.30 mg/kg; Zn = 14.60 mg/kg), total phenolic compounds (537.40 mg GAE/100 g), and demonstrates antioxidant capacity (DPPH inhibition activity = 96.75%). Due to the high nutritional value, bioactive compound content, and antioxidant properties of cauliflower stems, their valorization is of significant interest.

This paper presents the nutrient content, bioactive compounds, and antioxidant capacity of a functional ingredient (powder) obtained through the valorization of cauliflower stems.

MATERIALS AND METHODS

Samples

Cauliflower stalks were procured from Romanian farmers (three farmers from Ialomița County). The experiments were carried out at IBA-Bucharest, within the Pilot Vegetable-Fruit Processing Experimental Station.

The technological process for obtaining the powder from cauliflower stalks includes the following technological operations: sorting, washing, cleaning, division, boiling in water at 98-100°C for 4-5 minutes, dehydration, grinding, and packaging.

The dehydration of the plant material was carried out with hot air at a temperature of 50°C, in a convective electric dryer (Memmert), on stainless steel trays, with a hot air speed of 2.3 m/s, for 15.5-16 hours, until a maximum moisture content of 8.5%.

The grinding of the dehydrated plant material was carried out using a Knife Mill Grindomix GM 200, at a temperature of 20-22°C, to prevent the degradation of bioactive compounds and the properties of the functional ingredient (powder). The functional ingredient (powder) was packaged in glass containers, protected with aluminum foil, and sealed hermetically using twist-off lids.

The Figure 1 show cauliflower stalks powder three samples, from three different sources (farmers).

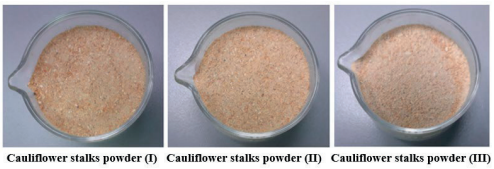


Figure 1. Cauliflower stalks powder from three different sources (farmers)

Statistical Analysis

The cauliflower stalks powder samples were analyzed in triplicate, and the results obtained are presented as the arithmetic mean and standard deviation.

Sensory analysis

The sensory analysis of the cauliflower stalks powder samples was carried out by evaluating the sensory characteristics (appearance, color, taste, and odor).

Instrumental analysis of color (L*, a* and b*) was performed with CM-5 colorimeter (Konica Minolta, Japan), using SpectraMagic NX software.

Physico-chemical analysis

The nutritional composition of the cauliflower stalks powder samples was determined using AOAC methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content), and 985.29 (total dietary fiber). The moisture content of the cauliflower stalks powder samples was determined using the Halogen

Moisture Analyzer HE53 (Mettler Toledo). Total carbohydrates were calculated with the formula: 100 (%) - moisture (%) - protein (%) - fat (%) - total dietary fiber (%). The energy value was calculated using conversion factors of 9 kcal/g for fat, 4 kcal/g for carbohydrates and protein, and 2 kcal/g for fiber, in accordance with Commission Regulation no. 1169/2011 (European Commission, 2011).

Determination of iron, copper, manganese, and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model NexION300Q, Perkin Elmer) after dry digestion of the samples. The determination of sodium, potassium, calcium, and magnesium was carried out by High-Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 - High-Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

Bioactive compounds content

The determination of the total polyphenol content was carried out using the Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, method and extraction time, volume of extract used in the analysis), using a UV-VIS Jasco V 550 spectrophotometer at a wavelength of λ = 755 nm. For quantification, a calibration curve of gallic acid in the concentration range of 0-0.20 mg/mL was used, and the results were expressed as mg of Gallic Acid Equivalents (GAE) per g of cauliflower stalks powder.

The determination of vitamin C content was carried out by liquid chromatography (Accela chromatograph, Thermo Scientific) coupled with high-resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) (Catană et al., 2017). The vitamin E (α-tocopherol) content was determined by chromatographic method (HPLC-DAD) (Popović et al., 2015). The content of glucosinolates was determined using a spectrophotometric method (Mawlong et al., 2017). The total carotenoid content was determined using a spectrophotometric method (Chinnadurai et al., 2013).

Antioxidant capacity

Antioxidant capacity was performed by the DPPH (1,1-diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, method and extraction time, volume of extract used in the analysis), using a UV-VIS Jasco V 550 spectrophotometer, at a wavelength of λ = 517. For quantification, a calibration curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) in the concentration range of 0-0,4375 mmol/L was used, and the results were expressed as μmol Trolox Equivalents per g of cauliflower stalks powder.

Microbiological analysis

The microbiological analysis of the cauliflower stalks powder was carried out using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 method (Enterobacteriaceae), ISO 21807:2004 (Water activity). Water activity was determined using Aquaspector AQS 31 equipment.

RESULTS AND DISCUSSIONS

Sensory analysis

The sensory analysis highlighted that the functional ingredient obtained from cauliflower stalks appears as a white-yellowish powder with a characteristic taste and smell typical for the Cruciferous family.

Following the instrumental color analysis (Figure 2), it was found that the powder obtained from the cauliflower stalks recorded high values of luminance L, ranging from 83.78 to 85.89. The b parameter varied between 21.01 and 22.87. The lowest values were recorded for the a* parameter (2.21-3.46). The luminance L* values recorded for the powder obtained from cauliflower stalks are significantly higher compared to those reported by Drabiańska et al. (2021) for the powder obtained from cauliflower leaves by lyophilization and grinding: L* = 43.51.

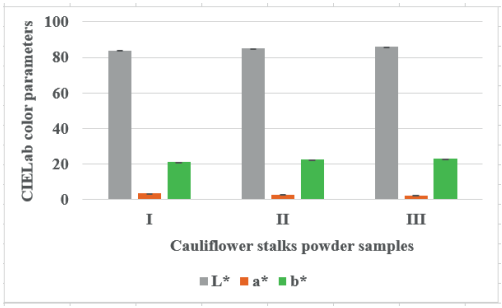


Figure 2. Colour parameters of the cauliflower stalks powder

At the same time, the values of the b parameter are 3.71-4.04 times higher compared to those reported by Drabiańska et al. (2021) for the powder obtained from cauliflower leaves by lyophilization and grinding: b = 5.66.

Physico-chemical analysis

The physico-chemical composition of the cauliflower stalks powder is presented in Table 1. The cauliflower stalks powders stand out for their carbohydrate content (55.08-57.50%), available carbohydrates (29.21-31.81%), soluble sugars (14.32-16.73%), protein (22.33-24.09%), ash (9.12-9.73%), and total fiber (23.27-26.85%).

Table 1. The physico-chemical composition of the cauliflower stalks powder

Physico-chemical composition	Cauliflower stalks powder		
	Sample I	Sample II	Sample III
Moisture (%)	6.90±0.17	6.72±0.17	6.61±0.17
Ash (%)	9.12±0.14	9.45±0.14	9.73±0.15
Protein (%)	22.33±0.33	23.12±0.35	24.09±0.36
Fat (%)	4.15±0.05	4.28±0.06	4.49±0.07
Carbohydrates (%)	57.50±0.37	56.43±0.37	55.08±0.36
Available carbohydrates (%)	30.65±0.20	29.21±0.19	31.81±0.20
Soluble sugars (%)	14.32±0.09	15.65±0.10	16.73±0.11
Total fiber (%)	26.85±0.50	25.42±0.47	23.27±0.43
Energy value (kcal/100g)	303	306	311
Energy value (kJ/100g)	1269	1282	1303

The ash content of the cauliflower stalks powder obtained in this experimental study is lower compared to that reported by Drabińska et al. (2021), 11.78%, in the case of this powder. At the same time, the ash content of the cauliflower stalks powder obtained in this study is 2-2.13 times higher compared to that reported by Sharma and Prasad (2018) for this functional ingredient. The protein content of the functional ingredient (powder) obtained from cauliflower stalks is higher compared to that reported by Drabińska et al. (2021), 19.12%, and by Sharma and Prasad (2018), 17.02%, in the case of this powder. The cauliflower stalks powders are sources of protein, since, according to Regulation (EC) No 1924/2006 of the European Parliament and of the Council, at least 12% (in this case 29.48%-30.98%) of their energy value comes from proteins.

The lipid content of cauliflower stalks powder is comparable to that reported by Drabińska et al. (2021): 4.74%. The carbohydrate content of the cauliflower stalks powder is about 1.17 times higher compared to that reported by Catană et al. (2023) for the cauliflower leaves powder (46.93%-49.34%), and 1.07-1.11 times higher compared to that reported by Sharma and Prasad (2018) for cauliflower stalks powder (51.56%).

The cauliflower stalks powders are significant sources of fiber (23.27%-26.85%). The fiber content of the cauliflower stalks powders obtained in our research is 1.45-1.68 times higher than that reported by Sharma and Prasad (2018) for this functional ingredient. At the same time, the fiber content of the cauliflower stalks powders is lower compared to that reported by Catană et al. (2023) for the cauliflower leaves powder (25.11%-30.45%). International studies demonstrate the importance of fiber in a healthy diet and its beneficial effects on the human body. Thus, fibers have water-holding capacity, reducing food intake (Dhingra et al., 2012; Liu et al., 2020), gelling capacity, increasing satiety (Wanders et al., 2013), glucose absorption capacity, lowering blood glucose (Liu et al., 2020), cholesterol-binding capacity, lowering serum cholesterol (Massa et al., 2022), antitumor capacity (Tang et al., 2024), and immunomodulatory activity (Dong et al., 2023; Tang et al., 2024). Given the beneficial effects of fibers on the human body, fortifying bakery and pastry products with cauliflower stalks powders is of real interest.

The mineral content of the cauliflower stalks powders is presented in Figures 3, 4, and 5. Potassium has the highest content among the analyzed mineral elements, ranging from 1785.35 to 2175.23 mg/100 g (the minimum value was recorded in sample I, and the maximum value in sample III)."

The potassium content of the cauliflower stalk powders is 1.92 to 2.16 times lower compared

to that reported by Catană et al. (2023) for cauliflower leaf powder.

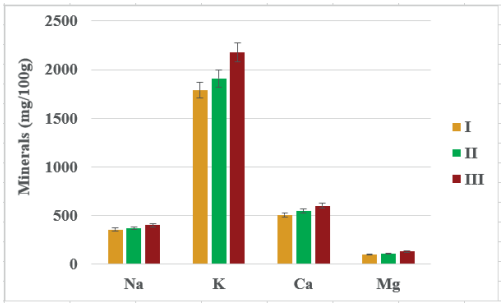


Figure 3. Mineral content (Na, K, Ca, and Mg) of the cauliflower stalks powders

Potassium is the main intracellular cation in the body and is particularly involved in membrane potential and electrical excitation of both nerve and muscle cells, as well as in acid-base regulation. Increased potassium intake lowers blood pressure, and this effect is especially important in hypertensive individuals (Frassetto et al., 2023).

Given these positive effects of potassium, fortifying food products (especially bakery and pastry products) with functional ingredients rich in potassium is of real interest.

Additionally, the cauliflower stalk powders have a high calcium content: 505.35-598.86 mg/100 g. By fortifying food products with this functional ingredient, their calcium content is increased.

Calcium is an important mineral that plays an essential role in human health, especially in bone health (Xu et al., 2020).

Calcium deficiency can lead to conditions such as osteoporosis, rickets, epilepsy, and anemia (Shojaeian et al., 2019).

The magnesium content of the cauliflower stalk powders ranged from 97.56 to 128.56 mg/100 g, being 2.69 to 3.54 times higher compared to the magnesium content in cauliflower by-products powder: 36.3 mg/100 g (Tukassar et al., 2023).

The iron content of the cauliflower stalk powders ranged from 28.43 to 43.57 mg/100 g, being 1.62 to 2.48 times higher than the iron content reported by Sharma and Prasad (2018) for this functional ingredient.

The iron content of Sample III from this experimental study is comparable to that reported by Catană et al. (2023) for cauliflower leaf powders, Sample I: 40.75 mg/100 g.

Iron plays a major role in the proper functioning of the human body. It is vital in oxygen transport, being the main component of hemoglobin and myoglobin, and also plays a role in oxidative phosphorylation.

Iron deficiency leads to iron-deficiency anemia and may contribute to cardiovascular disease, cancer, and developmental disorders of the brain and central nervous system (Samarakoon et al., 2023).

Therefore, fortifying food products with iron from natural sources is important.

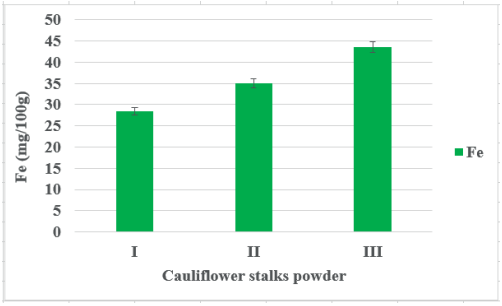


Figure 4. Iron content of the cauliflower stalks powders

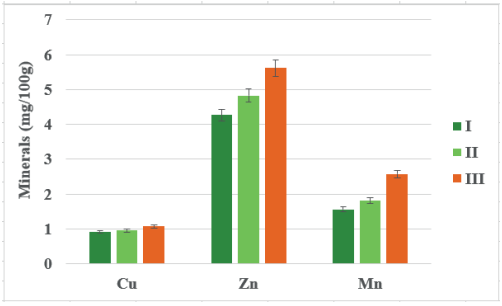


Figure 5. Mineral content (Zn, Cu and Mn) of the cauliflower stalks powder

The zinc content of the cauliflower stalk powder ranged between 4.27 and 5.62 mg/100 g. The manganese and copper content of the functional ingredient obtained from the valorization of cauliflower stalks is lower compared to the zinc content (Mn: 1.56-2.58 mg/100 g; Cu: 0.92-1.07 mg/100 g). The manganese content of the cauliflower stalk powders is significantly higher compared to that reported by Saleh (2022) for cauliflower waste powder (0.14 mg/100 g).

Bioactive compounds content

Cauliflower stalk powders exhibit a notable biochemical composition, characterized by their content of bioactive compounds (total polyphenols: 4.52-5.47 mg GAE/g d.w.; glucosinolates: 7.28-8.20 µmol/g d.w.; vitamin C: 45.03-48.12 mg/100 g; α-tocopherol: 1.05-1.44 mg/100 g; total carotenoids: 0.287-0.305 mg/100 g).

The results are in agreement with those obtained by Tlais et al. (2020), who state that, in addition to glucosinolates, cauliflower by-products are also notable for their content of vitamin C, carotenoids, and phenolic compounds.

Table 2 presents the bioactive compound content of the cauliflower stalk powders.

Table 2. Bioactive compounds content of the cauliflower stalks powders

Bioactive compounds	Cauliflower stalks powder		
	Sample I	Sample II	Sample III
Total polyphenols (mg GAE/g d.w.)	4.52±0.11	5.36±0.13	5.47±0.14
Glucosinolates (µmol/g d.w.)	7.28±0.18	7.87±0.20	8.20±0.21
Vitamin C (mg/100g)	48.12±1.44	46.24±1.39	45.03±1.35
α-Tocopherol (mg/100g)	1.05±0.03	1.27±0.03	1.44±0.04
Total carotenoids (mg/100g)	0.287±0.002	0.295±0.003	0.305±0.003

The total polyphenol content of the cauliflower stalk powders obtained in this study is 1.70 to 2.06 times higher than that reported by Drabiańska et al. (2021) for fine powder obtained from lyophilized and ground cauliflower stalks (2.65 mg GAE/g d.w.). Moreover, the total polyphenol content in this study is comparable to the value reported by Tukassar et al. (2023) for cauliflower by-products powder (5.37 mg/g).

Polyphenols represent an important class of biochemical compounds with beneficial effects on human health. Epidemiological studies conducted worldwide have shown that moderate and long-term consumption of polyphenol-rich foods may help prevent the development of cancer and chronic diseases such as cardiovascular and neurodegenerative diseases, type 2 diabetes, and obesity (Bié et al., 2023).

Additionally, cauliflower stalk powders are notable for their glucosinolate content, ranging from 7.28 to 8.20 µmol/g d.w. This is lower than the levels reported by Catană et al. (2023) for cauliflower leaf powders (240.23-259.65 µmol/g d.w.).

Recent research supports the protective role of glucosinolates in chronic diseases through mechanisms involving modulation of oxidative stress, inflammation, and detoxification pathways. Upon consumption, glucosinolates undergo enzymatic hydrolysis by myrosinase, resulting in the formation of bioactive compounds such as isothiocyanates and specific degradation products of indole glucosinolates, such as indole-3-carbinol and 3,3′-diindolylmethane, which contribute to various health benefits including anticancer, anti-inflammatory, and cardioprotective effects (Baldelli et al., 2025).

The vitamin C content of the cauliflower stalk powders ranged from 45.03 to 48.12 mg/100 g, being 1.70 to 1.86 times lower than the content reported by Catană et al. (2023) for cauliflower leaf powders (76.50-89.44 mg/100 g). However, the vitamin C content found in this study is comparable to the value reported by Kaviyarasi and Subapriya (2024) for dried cauliflower leaves (52 mg/100 g).

Vitamin C is an important cofactor in collagen and hormone synthesis and is involved in immune function, iron absorption, and antioxidant-related processes. Furthermore, it plays a significant role in improving quality of life, preventing chronic diseases and cardiovascular conditions, and is used in treatments for infections, neurodegenerative diseases, and cancer (Alberts et al., 2025).

The vitamin E (α-tocopherol) content of the cauliflower stalk powders ranged from 1.05 to 1.44 mg/100g, which is comparable to the value reported by Catană et al. (2021) for elderberry pomace powder obtained through hot air drying at 50°C (Sample II = 1.50 mg/100 g).

α-Tocopherol is an important peroxyl radical scavenger, playing a key role in maintaining metabolic health in tissues (Traber, 2023).

Cauliflower stalk powders also have a low total carotenoid content, ranging from 0.287 to 0.305 mg/100g.

Antioxidant capacity

Due to their complex biochemical composition, the cauliflower stalk powders exhibit antioxidant capacity, ranging from 10.72 to 12.94 µmol Trolox Equivalents/g (Figure 6).

The antioxidant capacity of the cauliflower stalk powders is 1.10 to 1.25 times lower compared to that reported by Catană et al. (2023) for cauliflower leaf powders, which ranged from 13.36 to 14.2 µmol TE/g.

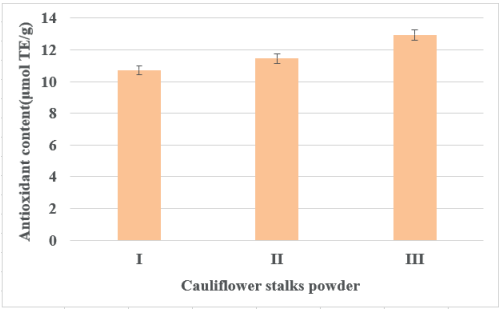


Figure 6. Antioxidant capacity of the cauliflower stalks powders

Microbiological analysis

The cauliflower stalks were processed under food-safe conditions, and the microbiological analysis of the cauliflower stalk powders showed that they comply with the current legal regulations (Table 3).

Table 3. Microbiological analysis of the cauliflower stalks powders

Microbiological indicators	Cauliflower stalks powder		
	Sample I	Sample II	Sample III
Yeast and molds (CFU/g)	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10
Water activity	0.266	0.257	0.246

The cauliflower stalk powders recorded low water activity values (0.246-0.266), which contributes to their quality stability in terms of sensory, physicochemical, microbiological properties, and bioactive compound content.

CONCLUSIONS

The research conducted in this experimental study revealed that cauliflower stalks possess a complex biochemical composition, and their valorization in the form of powders is of significant interest. Thus, based on a proprietary technology and under food-safe conditions, the cauliflower stalks were processed into powders with high nutritional value and notable antioxidant capacity.

The cauliflower stalk powders stand out for their content of protein (22.33-24.09%), ash (9.12-9.73%), available carbohydrates (29.21-31.81%), soluble sugars (14.32-16.73%), and total fiber (23.27-26.85%).

Additionally, the cauliflower stalk powders are rich in minerals, particularly potassium, calcium, magnesium, iron, and zinc (K: 1785.35-2175.23 mg/100 g; Ca: 505.35-598.86 mg/100 g; Mg: 97.56-128.56 mg/100 g; Fe: 28.43-43.57 mg/100 g; Zn: 4.27-5.62 mg/100 g).

These powders are also valuable due to their bioactive compound content (total polyphenols: 4.52-5.47 mg GAE/g d.w.; glucosinolates: 7.28-8.20 µmol/g d.w.; vitamin C: 45.03-48.12 mg/100 g; α-tocopherol: 1.05-1.44 mg/100 g; total carotenoids: 0.287-0.305 mg/100 g). Moreover, the functional ingredient obtained from cauliflower stalks exhibits the ability to neutralize free radicals, with an antioxidant capacity ranging from 10.72 to 12.94 µmol Trolox Equivalents/g.

Due to their high nutritional value, rich bioactive compound profile, and antioxidant capacity, cauliflower stalk powders represent valuable functional ingredients that can be successfully used in the fortification of food products.

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TRACING MINERAL PATHWAYS IN HALOPHYTES: FROM ROOTS TO AERIAL PARTS

Carmen Gabriela CONSTANTIN[1], Aurora DOBRIN[1], Andrei MOȚ[1], Andrei PETRE[1], Mihaela Maria ZUGRAVU[1], Mugurași Florin CONSTANTIN[2], Maria Marilena PARASCHIV[2, 3], Ioana-Cătălina NICOLAE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]National Institute of Research and Development for Biological Sciences of Bucharest, 296 Splaiul Independenței, District 6, Bucharest, Romania
[3]National University of Science and Technology Politehnica Bucharest, 313 Splaiul Independenței, District 6, Bucharest, Romania

Corresponding author email: catalina.nicolae@qlab.usamv.ro

Abstract

Soil salinization is a growing global challenge, affecting agricultural productivity and ecosystem stability. This phenomenon results from both human activities, such as improper irrigation and deforestation, and climate change-driven factors like rising temperatures and altered precipitation patterns. With increasing soil degradation, effective and sustainable solutions are urgently needed.
Halophytic plant species offer a promising strategy for mitigating soil salinization. These plants can naturally tolerate high salt concentrations by absorbing and transporting excess salts to specific tissues, thereby reducing soil salinity. Additionally, halophytes hold economic potential, as they can be utilized in various industries, including bioenergy, pharmaceuticals, and agriculture. Some species provide alternative food sources or raw materials for cosmetics and medicine.
By integrating halophytes into land management practices, degraded soils can be rehabilitated while generating valuable resources. However, further research is needed to optimize species selection and large-scale implementation. This study highlights the extraction potential of halophytes in addressing soil salinization and emphasizes their role in sustainable land use strategies.

Key words*: elemental analysis, inductively coupled plasma with mass spectrometry, phytoremediation, salinization.*

INTRODUCTION

To meet the growing global demand for food, one of the most effective solutions may be to increase land productivity. However, agricultural intensification involves high costs due to high water, energy, and pesticide consumption, as well as the depletion of soil nutrients and the negative impact on ecosystem services.
Poor management of agricultural practices increases soil salinity and water resource depletion, leading overall to the aridification of large areas. Soil salinity is a well-known abiotic stress factor that can hinder plant growth and development, reducing agricultural yield (Amombo et al., 2018; Barros et al., 2021; Rahman et al., 2021). One alternative solution may be to remediate these lands by using potential new crops, such as halophytes (Hasanuzzaman et al., 2014; Ahmadi et al., 2022). Through their morphological, physiological and anatomical characteristics, they have evolved the ability to tolerate high concentrations of sodium chloride (NaCl) (Rahman et al., 2021). Halophytes tolerate sodium (Na^+) and potassium (K^+) ions due to their capacity to sequester cytotoxic Na^+ in the vacuoles of root and leaf cells – a trait conferred by the constitutive expression of tonoplast Na^+/H^+ exchangers NHX complemented by the control of slow and fast vacuolar ion channels to prevent Na+ leakage back into the cytosol (Panta et al., 2014).
In this context, this paper presents a possible model of use of three halophyte species - *Portulaca oleracea, Festuca arundinacea Limonium sinuatum*, highlighting the

translocation of mineral elements from soils to different parts of them.

The World Health Organization has referred to purslane as a 'Global Panacea' and it is regarded as one of the most valuable medicinal herbs (Sultana & Rahman, 2013) due to its high content in vitamins, amino acids, minerals and polysaccharides (Petropoulos et al., 2016; Bekmirzaev et al., 2021; Jalali & Ghasemzadeh Rahbardar, 2022; Srivastava et al., 2023). Because of its remarkable ability to adapt and thrive under adverse environmental conditions and diverse climatic zones, it is an invasives species, with a cosmopolitan distribution. *Portulaca oleracea* evolved a unique type of photosynthesis as a response to drought and salt stress. Even though it is agreed that the species presents a C4 metabolism and the leaf structure displays a Kranz anatomy, with the mesophyll cells arranged to form a ring around the bundle-sheath cells, it can swith to CAM metabolism, even in the absence of photosynthetic stems, making it a CAM-facultative (D'Andrea et al., 2014; Sdouga et al., 2019; Wang et al., 2023). Purslane cultivated under high salinity stress accumulates proline, an osmoprotectants, and shows an enhanced capacity of the antioxidative system by increasing the activity of ROS - scavenging enzymes and lowering the lipid peroxidation (Yazici et al., 2007; Ocampo & Columbus, 2012). Studies have highlighted a high potential for the phytoremediation of contaminated soils, as the species is capable of hyperaccumulating and removing from the excess minerals (Bekmirzaev et al., 2021) and heavy metals (Negi, 2018; Elshamy et al., 2019; Londe et al., 2025).

Another species included in this study, *Festuca arundinacea* is a member of the Poaceae family, and, along with other members of the genus *Festuca* (*F. ovina* L., *F. rubra* L.), it is commonly used for lawns, due to its resistance to abiotic stress factors such as high temperatures, water deficiency and saline stress (Szekely-Varga et al., 2023). *Festuca arundinacea,* commonly known as 'tall fescue', even though is it not a true halophyte, it has a moderate resistance to salinity stress (Díaz et al., 2013; Kaplan et al., 2017; Batog et al., 2023). Moreover, additional to its use in lawns, it can be cultivated for biocomposite and bioethanol production (Batog et al., 2023).

Limonium (family Plumbaginaceae) is an almost globally distributed genus of salt-tolerant species, with its primary center of diversity located in the Mediterranean area (Malekmohammadi et al., 2017; Koutroumpa et al., 2018; Jēkabsone et al., 2023). Those species are commonly known as sea lavender. Research on different *Limonium* species have shown that salinity tolerance relies primarily on the active movement and buildup of ions within the leaves, accompanied by the production of proline and soluble sugars, which function as compatible solutes to maintain an osmotic balance (Al Hassan et al., 2016; González-Orenga et al., 2020). Moreover, the species of this genus possess salt glands that are also found in halophytic members of other families, such as Frankeniaceae and Tamaricaceae, making them capable of secreting various ions (Ca^{2+}, Na^{+}, K^{+}, HCO^{3-}, Cl^{-}) and trace elements (Ni, Pb, Mn, Fe, Cu, As, Cd, etc.) (Flowers et al., 2010; Caperta et al., 2020). The species used in this study, *Limonium sinuatum*, is commonly cultivated for its used as dried flowers (Xu et al., 2021). The dwarf varieties of *Limonium* are ideal for flower beds and borders, while the taller varieties are used in small groups and mixed borders, especially in areas with poor soils and reduced precipitation (Toma, 2009)

Therefore, the study aims to present the translocation of the elements from saline soils in different parts of those plant species during their lifecycle, for future applications in soil remediation and their subsequent recovery.

MATERIALS AND METHODS

Biological material

The biological material consisted of seeds of *Portulaca oleracea* subsp. *sativa, Festuca arundinacea* L., and *Limonium sinuatum* L. These were sown, to produce 4-week-old seedlings, which were then transplanted into the experimental plots in Tulcea (non-saline soil) and Dâmbovița (saline soil) areas of Romania.

Depending on the species, the following parameters were monitored in April, May and July in both experimental plots: biometric measurements (plant height, number of leaves, flowers, stems, tillers and inflorescences, elemental content (CHN and minerals) of both

plants and soil; as well as soil electrical conductivity and pH.

The determination of the soil reaction (pH) was carried out using a potentiometric method, by directly measuring the concentration of hydronium ions in the soil suspension, with a soil-to-water ratio of 1:2.5 (in accordance with standard SR ISO 10390:2015). For this, 10 g of soil were weighed into a 50 mL bottle, to which 25 mL of double-distilled water were added, forming an aqueous solution. The suspension was subjected to agitation for 1 hour on a horizontal shaker. After agitation, the sample was left to rest for 1 hour.

Before measurement, it was agitated again for 5 minutes. The pH was read directly in the sample in the suspension.

The determination of the specific electrical conductivity (EC) was carried out in accordance with the reference standard: - SR ISO 11265 + A1: 1998 Soil quality.

The soil sample was dried in the atmosphere and sieved through a 2 mm strainer. Ten grams of soil were weighed and 50 ml of ultrapure water were added. The samples were shaken for 1 hour at 15 rpm. After decantation, the suspension was filtered, and the electrical conductivity was measured directly in the prepared sample.

Elemental analysis (CHN) - preparation of soil and plant samples

Soil and plant samples were collected from the experimental plots, dried, and ground to a size of 250 µm. For sample analysis, tin capsules (8 x 5 mm) were used into which 0.01-0.02 g of each sample was weighted. The same protocol was applied for the plant samples.

For the calibration curve of the equipment, Cystine was used as a standard. The total nitrogen (N) and carbon (C) content of the cystine/standard were 11.64 and 29.95%, respectively. Five standards were weighed, of different concentrations ranging from 0.5 to 2.5 mg.

Sample analysis

Sample analysis was performed using the EA3100 elemental analyzer.

The mineral composition of the samples (both plat and soil) was determined according to the method described by Constantin et al., 2023.

Regarding the biometric measurements, they were carried out on a number of five plants per experimental variant. Thus, depending on the species, the following parameters were analyzed: plant height (H), number of leaves (Nf), number of floral stems, number of inflorescences, number of tillers and height of floral stem

RESULTS AND DISCUSSIONS

Results on the growth and development of Limonium sinuatum

Regarding the growth and development of *Limonium sinuatum* in the two experimental lots, the results are included in Figure 1.

In terms of plant height, the growth on saline soil was comparable to the data recorded on the non-saline soil in Tulcea. Differences were relatively minor, up to 10 cm, in the last stage of data collection. Thus, in Tulcea, a height of approximately 50 cm was recorded, while 38 cm was recorded in the Dâmbovița area.

Concerning average leaves/plant, it can be seen that there were no major differences (10-11 leaves), during the first two months of the experiment. Nevertheless, this difference became significant in the last stage of data collection (38 leaves/plant in the Dâmbovița lot, compared to 86 leaves/plant in the Tulcea reference plot).

The formation of floral stems began in July in both experimental lots, with their number increasing on average to 10 and 12 stems/plant in the Dâmbovița and Tulcea plots, respectively. The height of the floral stem was recorded in both experimental plots, increasing on average to 26 and 28 cm.

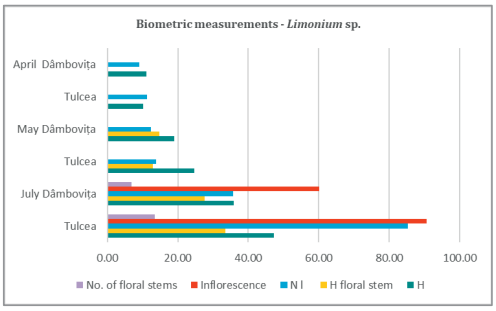


Figure 1. Dynamics of growth and development parameters of *Limonium* sp.

Similarly, the number of flowers grouped in inflorescences was counted in July, averaging between 60-83 flowers grouped in

inflorescences/plant, the differences being significantly different between the two plots.

Results on the growth and development of Festuca arundinacea

Regarding the growth and development of *Festuca* sp. in the two experimental plots, the results are presented in Figure 2. Therefore, regarding:

Plant height (H) - it can be observed that the species developed similarly in both experimental plots, with the plant height reaching a maximum of 27.4 cm;

Number of leaves (Nl) - the average is similar in the two experimental plots in the first two months, reaching a maximum number of 4 leaves in the case of plants from the Dâmbovița experimental plot;

The number of tillers (siblings) - similar to the plant height, this trait/ parameter did not differ significantly from the values recorded in the experimental plot in Tulcea.

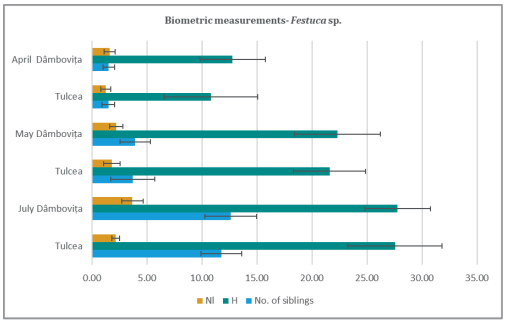


Figure 2. Dynamics of growth and development parameters of *Festuca* sp.

***Results on the growth and development of Portulaca oleracea* sp.**

Regarding the growth and development of the *Portulaca* sp. species in the two experimental batches, the results are shown in Figure 3. Therefore, regarding:

Plant height (H) - Compared to the data recorded on the non-saline soil in Tulcea, the height was generally similar. However, in May, the maximum height recorded in the Tulcea plot was 48 cm, while in the Dâmbovița plot it reached only 20 cm;

Number of leaves (Nl) - in the first two months, the number of leaves was similar (15-16 leaves). However, notable differences appeared in the final month (18 leaves Dâmbovița lot compared to 108 in the Tulcea reference plot);

Number of inflorescences - the formation of buds began in May in the Dâmbovița area, while in Tulcea in occurred during the months of June and July, likely due to geographical differences. However, on the saline soil the number of inflorescences continued to increase to an average number of approximately 37 per plant.

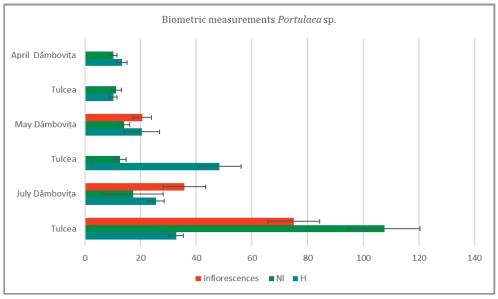


Figure 3. Dynamics of growth and development parameters of *Portulaca* sp.

All three species have showed, at least in terms of height, a stunned growth in the saline-soil plot in Dâmbovița, compared to the Tulcea plot.

Results of the analysis of ~~the~~ mineral composition in plants and soil

The results of mineral analysis of the plants grown in the experimental plot in Dâmbovița are presented in Table 1, while those from the experimental lot in Tulcea are shown in Table 2.

Table 1. Results regarding the mineral content of cultivated plants – experimental plot Dâmbovița

Sample	Na g/Kg	SD	Mg [g/Kg]	SD	K [mg/Kg]	SD	Ca [mg/Kg]	SD
Limonium leafs	34.93	1.63	4.812	0.210	12.35	0.64	6.46	0.57
Limonium roots	14.37	0.86	2.774	0.094	17.32	1.18	3.23	0.10
Festuca leafs	26.62	1.38	2.672	0.067	4.51	0.18	6.90	0.63
Festuca roots	10.05	0.43	1.635	0.038	5.60	0.42	6.77	0.60
Portulaca leafs	53.75	2.47	9.908	0.443	17.46	0.99	21.64	1.06
Portulaca stems	46.35	1.95	4.098	0.175	41.99	1.69	10.61	0.56
Portulaca roots	44.62	1.99	6.489	0.277	43.81	1.99	7.95	0.55

By comparing the results from the two experimental lots, the following observations can be made:

- High concentrations of sodium were found in the leaves of *Limonium* sp. and in all parts of *Portulaca* sp., especially in the leaves;
- The highest concentrations of magnesium were detected in the leaves of *Portulaca* sp.;
- *Portulaca* sp. also contains the highest concentration of potassium - 40 mg/kg in roots and stems; Simillarily, calcium is also found in large quantities in the leaves.

Within the Tulcea experimental group, the results indicate that the stems of *Limonium* sp. and the roots of *Portulaca* sp. are rich in sodium, even when grown in non-saline conditions. Similar levels of magnesium were also determined in the leaves and stems of the same species. The highest concentrations of potassium (77 g/kg) and calcium (20 g/kg) were found in stems of *Portulaca* sp.

Table 2. Results regarding the mineral content of cultivated plants – Tulcea experimental plot

Planta	Na [g/Kg]	SD	Mg [mg/Kg]	SD	K [g/Kg]	SD	Ca g/kg	SD
Limonium leafs	12.36	0.79	10.21	0.64	34.65	2.57	9.64	0.74
Limonium roots	2.21	0.10	2.08	0.10	21.51	1.50	2.08	0.13
Festuca leafs	1.29	0.06	2.91	0.12	23.80	1.41	4.24	0.15
Festuca roots	1.10	0.04	1.65	0.06	7.46	0.59	10.22	0.47
Portulaca stems	4.67	0.31	11.64	0.72	77.38	4.39	20.00	0.91
Portulaca roots	12.81	0.64	6.36	0.32	19.53	1.10	8.98	0.62

The results of soil analysis from the Dâmbovița and Tulcea experimental plots, before and after planting halophyte species, are presented in Table 3.

Table 3. Results regarding the analysis of minerals from cultivated soils

Soil sample	Na g/kg	SD	Mg g/kg	SD	K g/kg	SD	Ca g/kg	SD
Soil *Festuca* sp. Tulcea	0.13	0.00	6.240	0.080	3.29	0.30	29.16	0.79
Soil *Portulaca* sp. Tulcea	0.14	0.00	5.600	0.024	3.55	0.20	17.61	0.44
Soil *Limonium* sp. Tulcea	0.11	0.00	5.255	0.066	2.77	0.13	18.44	0.63
Initial soil Tulcea	0.08	0.00	5.243	0.028	2.93	0.12	18.07	0.57
Soil *Festuca* sp. Dâmbovița	1.54	0.05	12.424	0.113	2.00	0.11	31.68	0.62
Soil *Portulaca* sp. Dâmbovița	1.87	0.06	12.515	0.142	1.98	0.11	31.34	0.35
Soil *Limonium* sp. Dâmbovița	0.98	0.03	12.240	0.098	2.23	0.09	30.26	0.59
Initial soil Dâmbovița	2.05	0.05	12.544	0.081	2.27	0.08	29.76	0.45

Within the experimental plot in Dâmbovița, the results show a significant decrease in sodium concentration when cultivating *Limonium* sp., from 2 g/kg to 0.98 g/kg. In the case of calcium, a notable increase in concentration is observed only when cultivating the *Festuca* sp. Regarding magnesium and potassium, cultivation of the species in different conditions did not result in significant changes in their concentrations.

Within the experimental plot in Tulcea, according to the results obtained, significant changes were recorded in the calcium concentration when using *Festuca* sp.

Results on C, H, N analysis of soils and plants

The results of the C, H, N analysis for the soils and plant samples in the experimental plots are presented below in the form of tables and graphs.

The soil analysis results are presented in Table 4. According to the results obtained, a decrease in the amount of C, H, N compared to the initial (pre-planting) soil can be observed, demonstrating the hypothesis that the plants extracted these elements from the soil as they grew and developed.

Table 4. The C, H, N content of the soil in the Dâmbovița area

Sample	N %	C %	H %
Initial soil Tulcea	0.09 ± 0.06	1.21 ± 0.73	0.25 ± 0.15
Soil after cultivation Limonium sp.	0.12 ± 0.01	1.55 ± 0.07	0.31 ± 0.02
Soil after cultivation Portulaca sp.	0.15 ± 0.06	2.16 ± 1.04	0.42 ± 0.22
Soil after cultivation Festuca sp.	0.11 ± 0.06	1.62 ± 0.76	0.29 ± 0.14
Initial soil Dâmbovița	0.064 ± 0.01	1.608 ± 0.13	0.269 ± 0.02
Soil after cultivation Limonium sp.	0.054 ± 0.01	1.540 ± 0.03	0.227 ± 0.04
Soil after cultivation Festuca sp.	0.031 ± 0.02	1.495 ± 0.04	0.199 ±0.02
Soil after cultivation Portulaca sp.	0.051 ± 0.00	1.528 ± 0.07	0.197 ±0.03

For each species of plant, a detailed analysis of the C, H, N content found in different organs was made, as follows:

Festuca (root and leaves);

Portulaca (root, stems and leaves);

Limonium (root and leaves).

For *Portulaca* sp., the highest N content was determined in the shoots from the experimental lot in Dâmbovița. The highest value for C - 39.8% - was found in the roots from Tulcea, while the highest hydrogen (H) content, of 5.3%, was also determined at root level in the plants cultivated in Tulcea. In case of *Festuca arundinacea,* a carbon (C) content of 3.6% C was determined in the leaves of the plants cultivated in Dâmbovița. The C% content of in the roots was 42.9% and 42.8% in the Tulcea and Dâmbovița plots, respectively. Similar amounts of H were found in both roots and

leaves for of Festuca arundinacea cultivated in the two experimental plots.

Results of the analysis of soil pH and electrical conductivity

Results of pH and electrical conductivity measurements of soil samples from the experimental plots in Dâmbovița and Tulcea are presented in Figure 4.

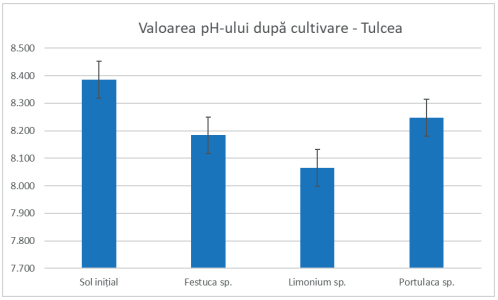


Figure 4. The pH and conductivity values of the soil samples before and after the cultivation of the species

According to the graph, it can be seen that in both experimental plots the pH value is modified after planting the three halophyte species, with the most notable changes occurring in the plots with *Festuca arundinacea*. These results are similar to those reported by Wang et al., 2021, who observed/ found that the cultivation of halophytes can reduce both soil pH and total salt content.

CONCLUSIONS

Sodium is the dominant element that influences the dynamics of minerals in the case of the experimental plot from Dâmbovița, where the soil salinity is significantly higher compared to the Tulcea plot. On the other hand, compared while the soil from Tulcea is clayey - rocky, the clayey-sandy soil structure from Dâmbovița Clayey-sandy soils allows a good preservation of moisture within the 10-30 cm soil depth range. The salt stress conditions in Dâmbovița induces a sodium dynamic exchange between the soil and the plants, with a significant decrease in soil salinity following cultivation of the *Limonium sinuatum.*

Overall, these results indicate a general trend in plants: higher concentration of the elements Na, Mg and Ca in the leaves compared to the roots, while the potassium concentration is slightly higher in the root.

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REMOVAL OF HEAVY METALS FROM WASTEWATER WITH THE HELP OF ADSORBENT MATERIALS OBTAINED FROM SLAG PRODUCED AS A RESULT OF STEELMAKING ACTIVITIES

Cristina-Ileana COVALIU-MIERLĂ, Maria RÂPĂ, Sorin IONESCU

National University of Science and Technology Politehnica Bucharest,
313 Splaiul Independenţei, 060042, Bucharest, Romania

Corresponding author email: cristina_covaliu@yahoo.com

Abstract

In addition to natural pollution, the environment is also affected by anthropogenic pollution, which has increased more and more with the development of industry and the excessive use of natural resources. A significant form of environmental pollution is heavy metal pollution. The most important sources of heavy metal pollution of the aquatic environment can be the discharge of domestic and industrial wastewater, agriculture and industrial processes. One way of removing heavy metals from wastewater is the application of adsorption processes using efficient adsorbents that are relatively easy to find, in large quantities and at a low price. These adsorbents can come from by-products obtained from industrial processes, such as slag resulting from the production of pig iron and steel. In this work, the use of blast furnace slag was followed to observe its ability to adsorb heavy metals from wastewater. The use of slag in the removal of heavy metals is a challenge and a way to research because there are multiple studies that provide evidence that slag can be used in wastewater treatment.

Key words*: slag, adsorbent material, wastewater.*

INTRODUCTION

A global problem is the pollution of water, surface and underground, and soil with heavy metals, which present a high toxicity and a slight biological enrichment, which makes them very dangerous for the environment and, implicitly, for human health (Ado-Ekiti et al., 2024). Heavy metals are known to be toxic and carcinogenic agents when discharged into the environment, which pose serious health problems and threaten flora and fauna in water bodies and soil (Ayesha Al Ali et al., 2021).

Chemical elements with an atomic weight between 63.5 and 200.6 and a specific gravity greater than 5 g/cm^3 are known as heavy metals (Ado-Ekiti et al., 2024). Heavy metals are ions of lead, cadmium, zinc, copper, cobalt, chromium, iron, nickel, arsenic, mercury, etc. Lead (Pb) is one of the most widespread pollutants that can accumulate quite easily in the human body, being non-biodegradable. The presence of lead in drinking water and food chains can cause neurological disorders, anemia, impairment of hemoglobin synthesis, cancer, kidney disease, nervous system damage, mental retardation, and gastrointestinal disease in the human body. Cadmium (Cd) is a dangerous heavy metal pollutant because cadmium is associated with health risks including kidney dysfunction, lung cancer, increased blood pressure, kidney failure, liver cirrhosis and bone atrophy. Zinc (Zn) is an important element for plant and animal growth, but higher content can lead to eye, nose, throat and skin irritation, stomach cramps and anemia. Copper (Cu) is a highly toxic element in drinking water, but less so than mercury. Excessive copper ingestion causes high blood pressure, anemia, liver and kidney damage, seizures, stomach and intestinal irritation (Ohidul Alam et al., 2024). Human health conditions due to long-term exposure to contact with heavy metals can be damage to nerves, muscles and physical processes, such as Alzheimer's disease, muscular dystrophy and Parkinson's disease and even lead to cancer. The high toxicity of these cationic heavy metals will cause significant damage to our lives if they are not kept under control and removed in time (Xiaofang Feng et al., 2022).

Heavy metal pollution is the result of industrial activities, improper waste disposal, and other man-made processes that release potentially toxic heavy metals into the environment. An-

thropogenic activities are responsible for the significant problem of heavy metal pollution in our water resources. Water quality is significantly degraded by the presence of toxic heavy metal ions, which is a serious threat because it makes water unusable for humans and animals (Nur Azalina Suzianti Feisal et al., 2024).

Heavy metals have received a lot of attention because they can come from many sources and show substantial toxicity, long persistence, non-degradability and easy bioaccumulation. Pollution with heavy metals occurs all over the world, which causes a high contamination of water bodies (Ohidul Alam et al., 2024).

Heavy metals, unlike organic pollutants, do not break down naturally and tend to accumulate in living organisms once they are introduced into the environment, so it is important that industrial effluents containing heavy metals are properly treated before they are released in the environment, to minimize their pollution and to protect both our natural environment and human health (Jonas Bayuo et al., 2024). The removal of harmful heavy metal ions from wastewater is crucial for protecting human health and the environment. By removing heavy metals from water, we can significantly reduce their harmful effects on the environment (Nur Azalina Suzianti Feisal et al., 2024).

Heavy metals in wastewater are strictly regulated by environmental protection authorities because of their harmful impact on health and toxicological properties. Useful technologies and materials must be developed for the removal of heavy metals from effluent waterways. The most widespread method of reducing and removing heavy metals from wastewater is adsorption (Niraj S. Topare, 2023).

Adsorption represents the retention of a matter or a substance at an interface, where the process takes place between a solid adsorbent and polluted water. It is a mass transfer progression that plays an important role in effluent treatment. The substance (pollutant) that is adsorbed is the adsorbate, and the adsorbing material is called the adsorbent. Adsorption is a surface phenomenon and occurs due to the weak Van der Waal's forces between the adsorbent and the adsorbate, resulting in a physical adsorption or due to the strong covalent bonds between the adsorbent and the adsorbate expressing chemical adsorption (Dawn S., Vinita V., 2021). Adsorption is a physico-chemical method that can be used to remove heavy metals from wastewater. Adsorption is flexible in design and operation, and cost and removal efficiency depend on the choice of adsorbent [Ayesha Al Ali et al., 2021].

Effective adsorbents should exhibit many beneficial properties, including high selectivity for rapid separation, thermal, chemical and mechanical stability, ability to be regenerated, low solubility in the contact liquid, large surface area, high efficiency, adequate pore size and volume, accessibility and low costs (Niraj S.T., Vinayak S.W., 2023).

Various parameters influence the adsorption process: contact time, pH of the solution, temperature of the solution, dosage of adsorbent, specific surface area of the adsorbent. Contact time has a significant effect on the adsorption, as the contact time increases, the adsorption rate also increases, after reaching the equilibrium, the removal rate is constant, as the time further increased, the adsorption rate is slowed due to the reduction of available active sites filled with heavy metal ions. Adsorbent dosage is a critical factor in controlling adsorption rates, it has been demonstrated that a small amount of adsorbent is sufficient to remove a reasonable concentration of heavy metals from contaminated media. When the pH of a negatively charged adsorptive surface is reduced, the surface is neutralized by the H^+ ions that are present in high numbers, enhancing the rate of adsorption. As the temperature increases, the rate of adsorbate diffusion across the external boundary layer also increases in the internal pores of the adsorbate. The adsorption rate directly depends on the surface area of the adsorbent: that is, the larger the surface area of the adsorbent, the high efficiency of adsorption. (Yared Daniel Retaa et al., 2023).

The removal of heavy metals using commercial adsorbent materials such as activated carbon is an expensive process, which has led researchers to find alternatives to commercial adsorbents. The adsorption mechanism, due to its simplicity, covers all types of adsorbents (Dawn S. and Vinita V., 2021). Including those obtained from waste and industrial by-products, such as blast furnace slag.

There are many studies and researches on the development of new and low cost adsorbents to remove heavy metals from wastewater by converting solid waste into good adsorption materials (Mengyu Ma et al., 2023), an example would be blast furnace slag that was used to remove heavy metals, as well as other pollutants, from wastewater: heavy metals: Hg^{2+}, Cr^{6+}, Pb^{2+}, Cu^{2+} (Alsulaili A. et al., 2023); heavy metals, phosphate and dyes (Abdelbasir M. et al., 2022); heavy metals like nickel, zinc, lead, chromium, copper, cadmium (Anjali M.S. et al., 2019); Pb(II), Cu(II), Zn(II), Cd(II), Ni(II) (Blahova L. et al., 2018); dyes, heavy metals like copper, nickel (De Gisi S. et al., 2016) etc.

Blast furnace slag is a by-product of pig iron production, it has a bulk density between 1200 and 1300 kg/m^3 and a specific gravity of about 2.90, having a complex chemical composition, showing, in particular, oxides such as SiO_2, Al_2O_3, Fe_2O_3, CaO and MgO Due to the crystalline structure that develops during the solidification process, blast furnace slag has the ability to adsorb pollutants.

Some research found that leaching from blast furnace slag was in the same range compared to leaching from untreated gravel, thus noting that neither the environment nor human health were adversely affected by this process.

Blast furnace slag presents a number of metal oxides with high porosity, specific surface area and strong adsorption characteristics, which can be successfully used as adsorbent material in wastewater treatment (J.N. Sahu et al., 2023).

MATERIALS AND METHODS

In this work, powdered blast furnace slag was used. The slag was passed through a set of sieves obtaining a granulometric distribution of 1 mm, 500 µm, 250 µm, 125 µm, 90 µm, 45 µm and a fraction below 45 µm. The slag fraction with the granulometry of 45 µm was collected, which is 16.15% of the slag taken into account.

An atomic absorption spectrometer contrAA 800 was used to perform the quantitative analysis of the slag sample. The solution of the sample to be analyzed is sprayed into the flame, taking place a series of processes through which free atoms are obtained. Atomic absorption spectrometry is a method based on the measurement of the absorption of electromagnetic radiation of a certain wavelength, by free atoms of the sample, in vapor state.

Previously, 3 solutions of different concentrations of 1 mg/L, 5 mg/L, 10 mg/L were prepared using 0.5% HNO_3 solution and multi-element solution of heavy metals to obtain the wastewater samples. A control sample was as well prepared, 0.5% HNO_3 solution, without pollutant. Also, 3 blast furnace slag samples were prepared, of 10 mg, 25 mg and 50 mg. The working times were 15 minutes, respectively 30 and 60 minutes.

The slag samples, in amounts of 10 mg, 25 mg and 50 mg, were immersed in 30 mL of heavy metal solution with a concentration of 5 mg/L. Magnetic stirring plates were used, at about 60 rpm, at room temperature, for 15 min, 30 min and 60 min. To remove the slag from the solution with heavy metals, the samples were centrifuged at 6 x 100 rpm for 10 min. For this work, the presence of heavy metals lead, cadmium, zinc and copper was monitored.

RESULTS AND DISCUSSIONS

The adsorption process is influenced by some important properties, such as pH, temperature, electrical conductivity (Table 1). The analysis of the amount of lead, cadmium, copper and zinc was carried out by atomic absorption spectrometry in flame. The analytical characteristics of the determination of the heavy metals analyzed in this work by atomic absorption spectrometry in the flame are shown in Table 2. Following the examination of the 10 mg, 25 mg and 50 mg samples with a flame atomic absorption spectrophotometer, the amounts of lead, cadmium, copper and zinc presented in Table 3 were found.

Table 1. The properties of the blast furnace slag sample

	pH, at 25°C	Electrical conductivity, µS/cm, at 25°C	Total dissolved solids content, mg/L
The sample of slag in water, 10%	8.9-10.3	72.4	39.7
The slag sample in the solution mixture containing pollutants	4.8-4.9	1000	531

Table 2. Analytical characteristics of the determination of heavy metals

Analytical characteristic / Metals / Heavy	Wavelength [nm]	Linearity of the curve, R^2	Limit of detection [mg/L]	Sensitivity of the method [mg/L/1% A]	Type of flame
Pb	217.0005	0.947614	2.081	0.059125	acetylene - air
Cd	228.8018	0.998584	0.3392	0.121037	
Cu	324.7540	0.994043	0.6963	0.023723	
Zn	213.8570	0.852891	3.545	0.027533	

Table 3. The amounts of heavy metals found in the analyzed samples

Heavy metals / Sample		S1 [mg]	S2 [mg]	S3 [mg]
Pb	15´	5.305	3.584	2.709
	30´	5.374	4.346	2.799
	60´	5.085	4.754	2.932
Cd	15´	6.827	6.844	6.945
	30´	6.805	6.960	6.902
	60´	6.678	7.109	7.234
Cu	15´	4.789	3.496	2.563
	30´	5.047	3.858	2.450
	60´	5.063	3.994	2.633
Zn	15´	7.102	7.032	7.075
	30´	7.119	6.999	6.890
	60´	7.100	7.182	6.983

The 10 mg sample was marked with S1, the 25 mg sample with S2, and the 50 mg sample with S3.
The contact time for each sample was 15 minutes (15'), 30 minutes (30') and 60 minutes (60').

In order to highlight the amounts of heavy metals (Pb, Cd, Cu, Zn) found in the analyzed samples, we made the graphs below (Figure 1).
In the case of the amount of Pb found in samples S2 and S3, an increase in the amount of Pb can be observed as the contact time increases, and in the case of sample S1, there is a decrease in the upward trend for a contact time of 60 minutes. Regarding the amount of Cd in the analyzed samples, in sample S1 a slight decrease is observed with the increase in contact time, in S2 there is an increase, and in S3 it goes through a slight decrease, as then at the time of 60 minutes to increase as the contact time increases. In the determination of the amount of Cu, an increase is observed in the case of samples S1 and S2, and in S3 there is a slight decrease and then an increase with the increase of the contact time. And in the case of the amount of Zn, there are changes for each sample like this, in S1 there is an increase and then a decrease, in S2 and S3 there is a decrease and then an increase with the increase in contact time. Thus, the influence of the amount of adsorbent and the contact time on the adsorption process can be observed.

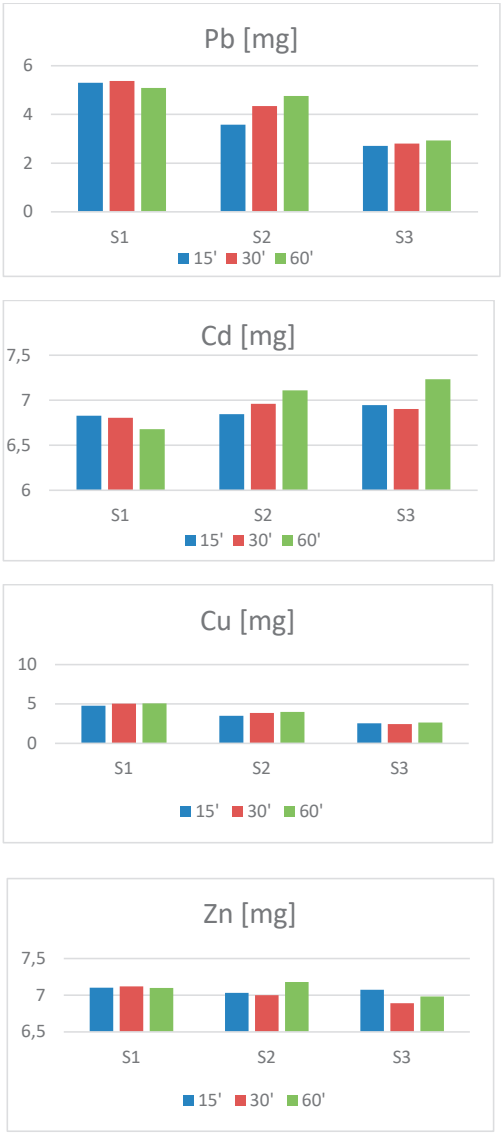


Figure1. The amounts of heavy metals found in the samples (a) lead, (b) cadmium, (c) copper, (d) zinc

CONCLUSIONS

In numerous researches, it is observed that blast furnace slag can be used in wastewater treatment as an adsorbent material for the removal of many pollutants, including heavy metals.

In this work, blast furnace slag, with a diameter of 45 µm, was used to observe its capacity to adsorb heavy metals from wastewater. The influence of the amount of adsorbent and the contact time on the adsorption process was also observed.

Blast furnace slag is an industrial by-product that can be used as an adsorbent in the removal of heavy metals, such as lead, cadmium, copper, zinc, from waste water.

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MEDIATION OR MODERATION: ANALYZING THE ROLE OF ENVIRONMENTAL PROTECTION IN THE RELATIONSHIP BETWEEN ECONOMIC GROWTH AND SOCIETAL HAPPINESS

Muhammad Ichsan FADILLAH, Dariusz PIEŃKOWSKI

Department of Law and Enterprise Management in Agribusiness, Faculty of Economics, Poznań University of Life Sciences, Ul. Wojska Polskiego 28 Poznań 60-624, Poland

Corresponding author email: ichsan.fadillah@up.poznan.pl

Abstract

This study examines the relationship between economic growth, represented by Gross National Income (GNI), environmental protection, measured by the Environmental Performance Index (EPI), and societal happiness, assessed through the World Happiness Report (WHR). Analyzing data from 122 countries over two years (2020 and 2022), the findings reveal that EPI partially mediates, and does not moderate, the positive effect of GNI on societal happiness. While GNI significantly correlates with happiness, improved environmental performance enhances this relationship. The study highlights the need for distinct policies addressing economic growth and environmental sustainability to effectively promote societal well-being. The findings reveal that environmental protection serves as a partial mediator in the relationship between GNI and WHR. While GNI shows a significant positive correlation with happiness, the quality of environmental performance also plays a crucial role in enhancing this relationship, suggesting that higher EPI scores contribute positively to societal happiness.

Key words*: economic growth, environmental protection, societal happiness, mediation, moderation.*

INTRODUCTION

The relationship between economic growth, environmental protection, and societal happiness have been a subject of various research in the past. However, the findings do not always come to an agreement with one another. There is research that puts more emphasis on environmental protection and happiness even if it means slow economic growth (Sekulova & van den Bergh, 2013). On the contrary, there is also research arguing that all three of these elements are interconnected, and therefore, none of them could be sacrificed for the other two (Cloutier & Pfeiffer, 2015).

There is also research that focuses only on environmental protection and societal happiness only, thereby excluding the perspective of economic growth altogether. A research found that the relationship between happiness and pro-environmental behaviours is cyclical (Nguyen et al., 2024). This means that individuals with higher happiness are more inclined to engage in pro-environmental behaviours, which in turn can enhance societal happiness by fostering a healthier and more sustainable environment (Nguyen et al., 2024). Similarly, another research highlighted that individual happiness is greater in natural environments, suggesting that preserving environments could contribute positively to societal happiness (MacKerron & Mourato, 2013).

Aside from economy, there is also research that associates the relationship between environmental protection and societal happiness with other aspects. A research found that environmental protection can play a significant role in enhancing happiness once it is coupled with social aspects such as family ties (MacKerron, 2011). On top of that, a research also argued that the aspect that can be correlated with environmental protection and societal happiness is social trust (Barrington-Leigh, 2017).

Regardless of what various past research has found in relation to environmental protection, the specific relationship between economic growth and happiness has been widely evidenced. A research found that economic growth and happiness is complex, suggesting that depending on income levels of the country, the relationship can be weak or strong (Zagórski et al., 2007). In poorer economies,

economic growth strongly correlates with happiness (Zagórski et al., 2007). In richer societies, however, the relationship is not as pronounced (Zagórski et al., 2007). This implies that there are factors that can influence this relationship. Given the extensive body of research on environmental protection side-by-side economic growth and happiness, it is reasonable to posit that environmental protection could be one of those factors.

This paper aims to investigate whether the role of environmental protection within the correlation between economic growth and societal happiness is moderating, fully mediating, or partially mediating. There are several ways how a third variable can impact the relationship between two variables of independent-dependent nature, which is moderation and mediation (Aguinis et al., 2017). Mediation itself can be further divided into two: full and partial (Aguinis et al., 2017). For moderation, it involves a variable that influences the strength or direction of the relationship between independent and dependent variables (Aguinis et al., 2017). For full mediation, it occurs when the effect of an independent variable on a dependent variable is entirely transmitted through a mediator (Aguinis et al., 2017). For partial mediation, it happens when the effect of an independent variable on a dependent variable is both direct (not mediated through a mediator) and indirect (mediated through a mediator) (Aguinis et al., 2017). In this case, the research question posed in this paper is: "Does environmental protection have moderating, fully mediating, or partially mediating effect on economic growth's relationship with happiness?"



Figure 1. Full Mediation

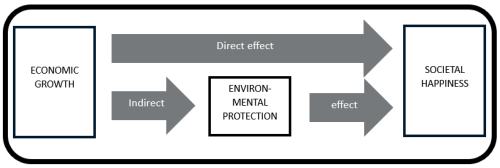


Figure 2. Partial Mediation

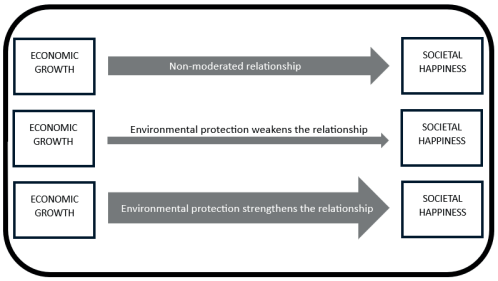


Figure 3. Moderation

MATERIALS AND METHODS

The three variables used in this paper are taken from various data sources. Economic growth is represented by the Gross National Income (GNI) that is taken from the World Bank (World Bank, 2024). The GNI data is PPP-adjusted (World Bank, 2024). Societal happiness is represented by the World Happiness Report (WHR) published jointly by Gallup, the Oxford Wellbeing Research Centre, the UN Sustainable Development Solutions Network, and the WHR's Editorial Board (World Happiness Report, 2024). Meanwhile, environmental protection is represented by the Environmental Performance Index (EPI) from the Yale Center for Environmental Law and Policy (Yale EPI, 2024). The EPI itself is a bi-annual data-driven summary of the state of sustainability that works as a scoring system for countries based on climate change performance, environmental health, and ecosystem vitality (Yale EPI, 2024).

The data included in this paper encompasses 122 countries in 2 years, 2022 and 2020. The countries are: Albania, Algeria, Argentina, Armenia, Australia, Austria, Azerbaijan, Bahrain, Bangladesh, Belarus, Belgium, Benin, Bolivia, Bosnia and Herzegovina, Botswana, Brazil, Bulgaria, Burkina Faso, Cambodia, Cameroon, Canada, Chad, Chile, China, Colombia, Comoros, Costa Rica, Croatia, Cyprus, Czechia, Denmark, Dominican Republic, Ecuador, El Salvador, Estonia, Ethiopia, Finland, France, Gabon, Gambia, Georgia, Germany, Ghana, Greece, Guatemala, Guinea, Honduras, Hungary, Iceland, India, Indonesia, Iraq, Ireland, Israel, Italy, Jamaica, Japan, Jordan, Kazakhstan, Kenya, Kuwait, Latvia, Lesotho, Lithuania, Luxembourg,

Madagascar, Malawi, Malaysia, Mali, Malta, Mauritania, Mauritius, Mexico, Moldova, Mongolia, Montenegro, Morocco, Mozambique, Myanmar, Namibia, Nepal, Netherlands, New Zealand, Nicaragua, Niger, Nigeria, North Macedonia, Norway, Pakistan, Panama, Paraguay, Peru, Philippines, Poland, Portugal, Romania, Rwanda, Saudi Arabia, Senegal, Serbia, Sierra Leone, Singapore, Slovenia, South Africa, Spain, Sri Lanka, Sweden, Switzerland, Tajikistan, Tanzania, Thailand, Togo, Tunisia, Uganda, Ukraine, United Arab Emirates, United Kingdom, United States, Uruguay, Uzbekistan, Zambia, and Zimbabwe.

The type of analysis done in this paper is statistical in nature. The analysis is done in R software. The steps of analysis are:

1. Simple linear regression of the original model (referred to as Model 1) to check the effect of GNI to WHR. The formula is as follows:

$$Model_1 = GNI \rightarrow WHR$$

Here, the estimate and the standard deviation of the GNI's coefficient is taken, as well as R^2 and P-value of Model 1,

1. Mediation analysis is done by using the following sub-steps:
   a. Simple linear regression of the mediation model (referred to as Model 2) to check the effect of GNI to EPI. The formula is as follows:

$$Model_2 = GNI \rightarrow EPI$$

   b. Like Model 1, here the estimate and the standard deviation of the GNI's coefficient is taken, as well as R^2 and P-value of Model 2,

   c. Multiple linear regression of the combined model (referred to as Model 3) to check the combined effect of both GNI and EPI as independent predictors of WHR. The formula is as follows:

$$Model_3 = GNI + EPI \rightarrow WHR$$

Here, the estimate and the standard deviation for each of the predictors' coefficients are taken, and so are the R^2 and P-value of Model 3,

   d. Calculation of indirect effect by multiplying the GNI's coefficient estimate of Model 2 and EPI's coefficient estimate of Model 3,

   e. Sobel test to determine the Z-value and P-value of indirect effect by using the following formulas:

   f. Z-value

$$z = \frac{a \,.\, b}{\sqrt{b^2 \,.\, SE_a^2 + \, a^2 \,.\, SE_b^2}}$$

where

$a = estimate\ of\ GNI's\ coefficient\ from\ Model\ 2$

$b = estimate\ of\ EPI's\ coefficient\ from\ Model\ 3$

$SE_a = standard\ deviation\ of\ GNI's\ coefficient\ from\ Model\ 2$

$SE_b = standard\ deviation\ of\ EPI's\ coefficient\ from\ Model\ 3$

   g. P-value

$$p = 2 \times (1 - pnorm\,(|z|))$$

where

pnorm is an R function that calculates the cumulative distribution function (CDF) for the standard normal distribution. It returns the probability that a standard normal random variable is less than or equal to the given value (in this case, z).

2. Moderation analysis using the built-in function in R's multiple linear regression of the moderation model (referred to as Model 4) as follows:

$$moderation = GNI * EPI \rightarrow WHR$$

This basically shows multiple regression analysis with 3 independent variables, which are GNI (independent prediction of GNI to WHR), EPI (independent prediction of EPI to WHR), and GNI:EPI (prediction of interaction term between GNI and EPI to WHR). The P-value of all three will be taken,

3. Determination of the significance by checking if all P-values in the analysis are less than 0.05.

RESULTS AND DISCUSSIONS

Below is the data used in this paper:

Table 1. Data used in this paper

Year		GNI	WHR	EPI
2020	Max	86,640	7.809	82.5
	Min	1,230	3.299	25.1
	Mean	23,031	5.625	49.7
2022	Max	118,470	7.821	77.9
	Min	1,210	2.995	18.9
	Mean	28,083	5.649	44.4

Below are the results:

Table 2. Results of regression analysis

Year	Models			Variables	Coefficient		
	Name	R^2	P-Value		Estimate	Standard Deviation	P-Value
2020	Model 1	0.6215	2.200e-16	GNI	4.074e-05	2.902e-06	2.000e-16
	Model 2	0.7561	2.200e-16	GNI	6.795e-04	3.524e-05	2.000e-16
	Model 3	0.6743	2.200e-16	GNI	1.983e-05	5.473e-06	0.00043
				EPI	3.076e-02	7.004e-03	2.450e-05
	Model 4	0.6766	2.200e-16	GNI	3.150e-05	1.384e-05	0.02460
				EPI	3.433e-02	8.014e-03	3.770e-05
				GNI: EPI	-1.882e-07	2.050e-07	0.3604
2022	Model 1	0.63930	2.200e-16	GNI	3.286e-05	2.253e-06	2.000e-16
	Model 2	0.57700	2.200e-16	GNI	3.844e-04	3.005e-05	2.000e-16
	Model 3	0.65920	2.200e-16	GNI	2.608e-05	3.381e-06	4.150e-12
				EPI	1.762e-02	6.681e-03	0.00946
	Model 4	0.66220	2.200e-16	GNI	3.690e-05	1.104e-05	0.00111
				EPI	2.366e-02	8.890e-03	0.00886
				GNI: EPI	-2.112e-07	2.051e-07	0.30525

Table 3. Results of indirect effect calculation and sobel test analysis

Year	Indirect Effect	Sobel Test	
		Z-Value	P-Value
2020	0.00000677	2.583004	0.009794409
2022	0.00002089	4.282109	0.000018513

The results of this study provide compelling evidence for the role of EPI in the relationship between GNI and WHR. The analysis across two years (2020 and 2022) reveals that while a significant relationship exists, it demonstrates partial mediation rather than full mediation nor moderation.

1. Presence of mediation

The results indicate that mediation is present due to the significant improvement in the R2 value from Model 1 (original model, GNI □ WHR) to Model 3 (combined model, GNI + EPI □ WHR). Specifically, the partial mediation is evidenced by the change in the GNI coefficient between these models. In Model 1, GNI has a direct positive effect on WHR (positive coefficient estimate of 4.074e-05 and P-value < 0.05), signifying that increases in economic growth correlate with enhanced societal happiness. When EPI is introduced in Model 3, the coefficient estimate for GNI decreases (to 1.983e-05) while maintaining statistical significance ($p < 0.05$). This suggests that part of the effect of GNI on

WHR is transmitted through EPI, supporting the idea that improvements in environmental performance contribute to societal happiness and therefore partially mediate the economic growth-happiness relationship.

While the GNI coefficient estimate lowers yet remains statistically significant from Model 1 to Model 3, the increase in R2 from Model 1 to Model 3 shows that mediation occurs because Model 3 can explain happiness better than Model 1. This improvement in explanatory power underscores the role of EPI in enhancing the understanding of how GNI influences WHR.

2. Evidence of partial mediation

The results support partial mediation rather than full mediation due to the continued significant direct effect of GNI on WHR. If EPI were a full mediator, the inclusion of EPI in Model 3 would nullify the direct effect of GNI on WHR, resulting in a non-significant GNI coefficient in that model. However, since GNI remains statistically significant in Model 3, it confirms that while EPI explains some of the variance in happiness, a direct path from GNI to WHR still exists, validating the notion of partial mediation.

Partial mediation can be clearly observed through the significance of both GNI and EPI coefficients in Model 3. For full mediation to occur, we would expect the EPI coefficient to remain significant while the GNI coefficient would lose its significance, which is not the case in our findings.

Furthermore, the calculation of the indirect effect, corroborated by the sobel test, shows a significant indirect effect ($p < 0.05$), which provides robust evidence for the mediating role of EPI. This significance of the indirect effect confirms the presence of partial mediation, as it indicates that the pathway from GNI to WHR through EPI is not only statistically relevant but also substantial. This finding effectively nullifies the possibility of full mediation, where one would expect the indirect effect to dominate entirely over the direct effect.

3. The direct and indirect effects

The analysis demonstrates that both the direct and indirect effects of GNI on WHR are statistically significant. The direct effect of GNI, as indicated in Model 1, shows a significant positive coefficient ($p < 0.05$), confirming that higher economic growth correlates directly with increased societal happiness.

Simultaneously, the sobel test results confirm that the indirect effect through EPI is also significant, with a P-value indicating robust mediation ($p < 0.05$). This significance reinforces the conclusion that environmental protection plays a vital role in enhancing societal happiness indirectly.

Furthermore, a comparison of the coefficients from Models 1 and 3 reveals that both the direct path (GNI to WHR) and the indirect path (GNI to WHR via EPI) retain their significance without one overpowering the other. The coefficients suggest that while the direct effect remains strong, the indirect effect is substantial enough to warrant attention, thereby confirming the presence of partial mediation. This interplay between the direct and indirect effects highlights the complexity of the relationship between economic growth, environmental performance, and societal happiness.

4. The lack of moderation

The lack of moderation is evident from the analysis of the interaction term (GNI: EPI) included in Model 4. The coefficient for the interaction term is not statistically significant ($p = 0.3604$), indicating that EPI does not influence the strength or direction of the relationship between GNI and WHR. This finding suggests that while environmental protection is related to societal happiness, it does not act as a conditional factor that alters the impact of economic growth on happiness. Consequently, we cannot assert that environmental performance modifies the effect of GNI on happiness, reinforcing the notion that the relationship is more direct than conditional.

5. The R^2 increase from model 1 to model 3 and model 1 to model 4

Despite the high R2 values and significant p-values across all models, the increase in R-squared from Model 1 to Model 3 and from Model 1 to Model 4 does not indicate that moderation occurs. The improvement in R-squared when introducing EPI into the GNI-to-

WHR relationship signifies that EPI accounts for additional variance in happiness beyond what is explained by GNI alone. This enhancement supports the idea that environmental performance plays a critical role in influencing societal happiness.

However, the absence of a statistically significant interaction term (GNI: EPI) in Model 4 indicates that EPI does not modify the effect of GNI on WHR. While the inclusion of EPI improves the model's explanatory power, it does so by acting as a mediator rather than a moderator. Therefore, the observed increases in R2 values illustrate the importance of considering environmental protection in understanding the relationship between economic growth and happiness, but they do not support the notion that moderation is present in the data.

These results ultimately illustrate that while environmental protection plays a role in mediating the relationship between economic growth and societal happiness, it does so partially and does not exhibit moderating effects. The significance of the indirect effect, as indicated by the Sobel test, further substantiates the notion of partial mediation and rules out full mediation. Future research could explore additional mediating variables or different methodologies to further elucidate the complexities of these relationships and refine our understanding of how economic, environmental, and societal factors interplay in influencing happiness.

6. Policy recommendations

The findings of this study highlight the critical role of environmental protection as a mediator in the relationship between economic growth and societal happiness. This mediation suggests that targeting economic growth and environmental protection separately, while acknowledging the influence of economic growth on environmental quality, is essential for enhancing overall happiness.

Governments should implement policies that stimulate economic growth through infrastructure investments and innovation support, directly correlating with societal happiness. Concurrently, stringent environmental regulations are necessary to improve environmental performance and, subsequently, happiness. Advancing renewable energy and sustainable practices as distinct initiatives further supports this goal.

Moreover, educational programs promoting economic skills and environmental awareness can prepare individuals to contribute meaningfully to both sectors. Policymakers should use separate data sets for each sector to inform strategies, ensuring targeted interventions. Public awareness campaigns that highlight the benefits of both economic growth and environmental health can also foster support for happiness.

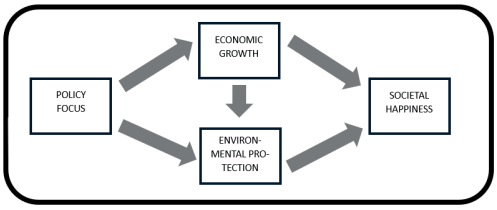


Figure 4. Recommended Policy Focus

In contrast, the lack of moderation role of environmental protections in economic growth's influence on happiness observed in this study indicates that merging economic growth strategies with environmental protections may lead to conflicting objectives and undermine effectiveness. Thus, combined policies should be avoided, as it would only detract from sustainability efforts and do not provide any added benefit to the positive impacts of economic growth on happiness.

In summary, effective policies should focus independently on economic growth and environmental protection while recognizing their interrelationship. By implementing these recommendations and steering clear of combined approaches, governments can create a framework that aligns with the study's findings, ultimately enhancing societal happiness.

CONCLUSIONS

This study investigates the interplay between economic growth, represented by Gross National Income (GNI), environmental protection, measured by the Environmental Performance Index (EPI), and societal happiness, assessed through the World

Happiness Report (WHR). The findings reveal that environmental protection serves as a partial mediator in the relationship between GNI and WHR. While GNI shows a significant positive correlation with happiness, the quality of environmental performance also plays a crucial role in enhancing this relationship, suggesting that higher EPI scores contribute positively to societal happiness.

Importantly, the analysis finds no evidence that EPI moderates the relationship between GNI and WHR. This indicates that policymakers should adopt distinct strategies for promoting economic growth and environmental sustainability, acknowledging their interdependence without conflating the two concepts. By implementing policies that separately address GNI growth and improvements in EPI, governments can foster a more effective approach to enhancing overall societal happiness. Future research should continue to explore the complexities of these relationships to further elucidate the dynamics at play.

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THE INFLUENCE OF HORTICULTURAL BY-PRODUCTS IN BAKERY PRODUCTS - A REVIEW

Diana-Alexandra GHEORGHIU[1], Liviu GACEU[2], Adrian PETICILĂ[1], Dana TĂPĂLOAGĂ[3], Nicoleta HĂDĂRUGĂ[1], Gabriela BRAD[1], Liliana TUDOREANU[1, 4]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Transilvania University of Braşov, 29 Eroilor Blvd, Braşov, Romania
[3]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului, Timişoara, Timiş, Romania
[4]Interdisciplinary Laboratory for Heavy Metals Accumulation in the Food Chain and Modelling, Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independenţei, District 5, Bucharest, Romania

Corresponding author email: diana-alexandra.gheorghiu@doctorat.usamv.ro

Abstract

Horticultural by-products (HBP) are valuable functional ingredients and natural preservatives that enhance the nutritional value and shelf life of foods. The review includes articles examining HBP addition in bakery products. Following PRISMA Guidelines, 1,970 articles were identified, with 47 selected for analysis. These studies explored HBP's impact on phenolic content, physicochemical composition, colour, rheological, and sensory properties in bakery products. The published data suggests that increasing the concentration of HBP positively correlated with high phenolic content and antioxidant capacity in bakery products. The main limitation of the published data was that no articles were found to contain a complete characterisation of the products: physicochemical parameters, rheological parameters and sensory analysis. Moreover, no standardisation of rheological methods was identified. Further investigations into HBP properties and usefulness will help optimize formulations, meet consumer preferences and increase the use of HBP to produce more healthy and sustainable food.

Key words*: texture; sensory analysis; phenols; minerals; rheology.*

INTRODUCTION

Reducing food losses and waste is crucial in mitigating pressure on natural resources and fostering sustainable food systems (FAO, 2019). One approach involves valorising by-products to harness phenolic compounds for developing functional foods, with bakery products serving as effective carriers for fortification (Dziki et al, 2014; Echave et al., 2023; Krajewska & Dziki, 2023; Santos et al., 2022).

Methods for extracting valuable phenolic compounds from these by-products have been investigated, and their incorporation into baked goods has been shown to increase phenolic content and antioxidant capacity (Olaoye & Ade-Omowaye, 2011; Piasecka & Górska, 2021; Ramya et al., 2023; Ranasinghe et al., 2022). However, further research is needed to optimize formulations, align with consumer preferences, and ensure regulatory compliance, in order to enable the development of innovative and sustainable bakery products that address both nutritional and environmental goals.

This review focuses on potential functional ingredients from horticultural by-products incorporated to bakery products.

Physicochemical characterization of the functional ingredients, and their effect on dough and final products properties will be summarized, but due to the wide variety of these by-products existing in literature, they were organized according to their origin in four categories: peels and skins; pomace; seeds and petals, leaves, stems and other floral by-products.

MATERIALS AND METHODS

Search Strategy

This review was conducted based on an extensive literature search across three major databases, following PRISMA 2020 guidelines to ensure rigor and minimize bias. The search focused on studies that investigated the influence of horticultural by-products on the quality, physicochemical, sensorial and rheological properties of bakery products, published in peer-reviewed English journals.

The initial search yielded 1,970 publications. After excluding 636 duplicates, 1,334 potentially relevant titles were identified. Screening of titles and abstracts resulted in 132 articles, and subsequent full-text screening led to the final inclusion of 47 articles.

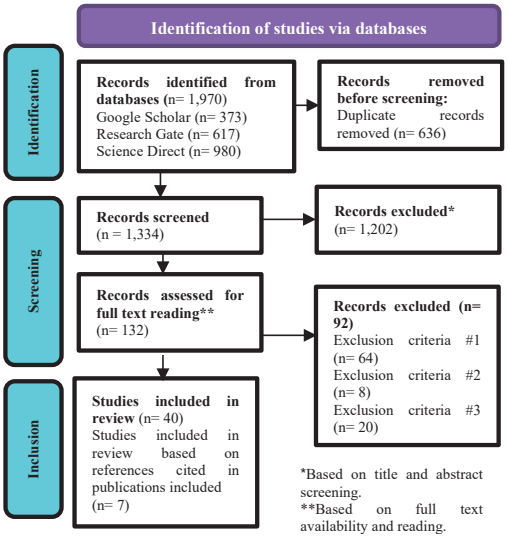


Figure 1. PRISMA flow diagram
(From: Page M.J. et al., 2021)

RESULTS AND DISCUSSIONS

Composition of Potential Functional Ingredients Obtained from Horticultural By-Products

Horticultural by-products, such as pomace, peels, seeds, and leftover fruits and vegetables, typically make up 20% to 45% of total produce. These by-products are rich in dietary fiber, polyphenols, antioxidants, essential fatty acids, and carotenoids. Incorporating them into baked goods can enhance the nutritional profile, sensory characteristics, and shelf life. This has been shown to significantly improve the nutritional and functional properties of products like cakes, muffins, cookies, crackers, and bread. It can also modify the texture, flavour, and overall nutritional composition, making the final products more appealing to consumers (Gómez & Martinez, 2018; Kauser et al., 2024; Kuyu, 2015; Sahni & Shere, 2019; Melini et al., 2020).

The composition of different horticultural by-products used in bakery products is summarized in Table 1.

The total phenolic content (TPC) varies widely, with peels and skins ranging from 2.14 to 6.47 g kg^{-1}, pomace from 10.16 to 45.75 g kg^{-1}, and petals, stems, leaves, and other floral by-products from 0.56 to 537.40 g kg^{-1} (Upadhyay et al., 2023; Sudha et al., 2007; Fardaghi et al., 2020; Tukassar et al., 2023). Analyses of pomace indicate protein is generally below 10 g kg^{-1}, except for mushrooms which are higher (Djeghim et al., 2021; Sudha et al., 2007; Upadhyay et al., 2023; Lu et al., 2018). Fat and ash levels are below 7 g kg^{-1} and 5 g kg^{-1}, while total dietary fiber (TDF) ranges from 10.15 to 49 g kg^{-1}. Other HBPs have fiber contents between 7.24 and 52.90 g kg^{-1}, with protein and fat levels generally ranging from 8.58 to 30.60 g kg^{-1} and 1.03 to 31.83 g kg^{-1}, respectively (Djeghim et al., 2021; Upadhyay et al., 2023; Segura-Badilla et al., 2022 Ojeda et al., 2023; Matseychik et al., 2021; Abdel-Hameed et al., 2023; Cerda-Bernad et al., 2023; Drabińska et al., 2018; Vinod et al., 2023). Mineral analysis shows potassium is particularly high in saffron stigmas and chickpeas, while iron levels in saffron stigmas are lower than cauliflower by-products and chickpeas (Cerda-Bernad et al., 2023; Tukassar et al., 2023; Vinod et al., 2023).

Table 1. Composition of potential functional ingredients recovered from horticultural by-products incorporated in bakery products

HBP	TPC	Moisture	Protein	Fat	Ash	TDF	Energy value (Kcal/100g)	Carbohydrate	Macrominerals			Microminerals					References
									Ca	Mg	Na	K	Fe	Mn	Zn	Cu	
PEELS AND SKINS																	
Pepper peels	-	6.04	4.38	2.46	2.92	15.90	-	35.20	-	-	-	-	-	-	-	-	Djeghimet al. (2021)
Prickly pear peels	-	10.29	8.48	1.69	19.60	52.90	-	52.53	-	-	-	-	-	-	-	-	
Prickly pear seed peels	-	7.87	7.00	1.03	16.42	50.80	-	16.88	-	-	-	-	-	-	-	-	
Potato peel	2.14	11.44	2.17	2.42	2.92	8.15 ± 0.22	-	-	-	-	-	-	-	-	-	-	Upadhyayet al. (2023)
Bottle gourd peel	6.47	9.40	2.74	2.43	3.92	7.24 ± 0.13	-	-	-	-	-	-	-	-	-	-	
Banana peel	-	3.56	6.41	10.22	11.86	14.38	-	57.13	-	-	-	-	-	-	-	-	Segura-Badilla et al. (2022)
POMACE																	
Orange pomace	-	9.90	5.40	4.37	3.76	12.41	-	64.16	-	-	-	-	-	-	-	-	Djeghim et al. (2021)
Apple pomace	-	11.41	3.66	3.16	1.51		-	64.36	-	-	-	-	-	-	-	-	
Tomato pomace	-	5.23	7.87	3.31	3.61	49.00	-	27.08	-	-	-	-	-	-	-	-	
Apple pomace	10.16	10.80	2.06	2.70	0.50	51.10	-	-	-	-	-	-	-	-	-	-	Sudha et al. (2007)
Apple pomace	10.45	5.50	1.53	4.15	1.89	10.15	-	-	-	-	-	-	-	-	-	-	Upadhyay et al. (2023)
Indian gooseberry pomace	45.75	9.31	1.77	6.14	0.86	13.15	-	-	-	-	-	-	-	-	-	-	
Vinal (Neltuma ruscifolia) residue	-	8.90	10.20	2.40	4.50	21.20	-	53.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Shiitake mushroom	-	10.49	24.68	-	-	-	-	-	-	-	-	-	-	-	-	-	Lu et al. (2018)
Black ear mushroom	-	13.81	10.92	-	-	-	-	-	-	-	-	-	-	-	-	-	
Silver ear mushroom	-	11.83	10.88	-	-	-	-	-	-	-	-	-	-	-	-	-	
SEEDS																	
Amaranth seed	-		13.80	7.10	2.20	-	-	62.30	-	-	-	-	-	-	-	-	Matseychik et al. (2021)
Papaya seed	-	7.15	27.95	31.83	7.86	18.53	6.68	424.99	-	-	-	-	-	-	-	-	Abdel-Hameed et al. (2023)
Vinal (Neltuma ruscifolia) seeds	-	5.90	30.60	4.60	3.20	20.90	-	35.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Vinal (Neltuma ruscifolia) endocarp	-	8.90	11.80	2.10	3.90	38.60	-	35.00	-	-	-	-	-	-	-	-	
PETALS, LEAVES, STEMS AND OTHER FLORAL BY-PRODUCTS																	
Spanish saffron petals	-	6.42 - 5.52	8.58 - 8.68	5.81 - 4.56	8.39 - 4.89	26.59 - 22.56	369 - 381	70.80 - 76.35	415.20 - 112.60	120.30 - 103.30	9.00 - 9.20	1530 - 1450	46.26 - 6.38	2.51 - 0.95	3.89 - 2.10	0.57 - 0.55	Cerda-Bernad et al. (2023)
Spanish saffron stigmas	-	8.90	13.15	7.01	5.59	17.86	377.00	65.26	118.30	100.20	19.00	1114.00	5.45	1.15	2.65	0.35	
Iranian saffron stigmas	-	9.01	13.61	6.08	5.57	21.17	372.00	65.73	86.25	102.50	33.75	1136.00	12.28	1.30	2.13	0.25	
Greek saffron stigmas	-	10.74	12.29	6.22	4.81	17.64	369.00	65.94	123.30	88.70	33.25	971.30	8.13	1.25	1.98	1.63	
Saffron stigma	2.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Fardaghi et al. (2020)
Saffron stamen	1.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Saffron style	0.76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Saffron petal	1.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Saffron corm	0.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Broccoli leaves	-		28.99		10.65	-	-	-	-	-	-	-	-	-	-	-	Drabińska et al. (2018)
Cauliflower stems, stalks and leaves	537.40	5.50	22.80	2.95	5.81	15.32	-	47.62	-	363.00	-	-	200.30		14.60	8.30	Tukassar et al. (2023)
Vinal (Neltuma ruscifolia) pods	-	8.70	12.80	2.40	4.10	31.10	-	41.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Chickpea	-	-	22.40	60.69	2.82	17.40	-	57.80	45.00	166.00	-	846.00	4.86	-	-	-	Vinod et al. (2023)

The Influence of Fruit, Vegetable and Legumes Peels and Skins on Bakery Products

The formulations of breads and bakery goods incorporating potential functional ingredients from peels and rinds are presented in Tables 2 and 3.

Variation of the total phenolic content, chemical composition and mineral content

According to a study by Manzoor et al. (2024), bagels containing banana peel and lavender extract exhibited high TPC compared to control samples.

The incorporation of horticultural by-products increased protein levels in breads by 8% to 182% when compared to their respective control samples. However, a study conducted

by Upadhyay et al. (2023) found that protein content decreased by 70% to 76% in HBP-containing bread compared to the control. Fat content in wheat-based bakery products increased by 11% to 81% with HBP addition, except for banana peel bread where fat levels decreased by 41%. TDF values rose by 12% to 192% in HBP-containing bakery items compared to their controls, while carbohydrate content decreased by 4% to 9% for banana peel bread and biscuits, but increased by 7% in bread with potato and bottle gourd peels compared to their respective control samples (Manzoor et al., 2024; Segura-Badilla et al., 2022; Upadhyay et al., 2023). The reviewed studies did not examine the influence of adding HBP peels and skins on the mineral content of bakery products.

Table 2. Nutritional composition of bakery products with horticultural peels and skins incorporated in bakery products

HBP	Bakery product	% added	TPC	Moisture	Protein	Fat	Ash	TDF	Energy value (Kcal/100 g)	Carbohydrate	Macrominerals			Microminerals					Reference
											Ca	Mg	Na	K	Fe	Mn	Zn	Cu	
Banana peel and lavender	Bagels	10 5, 10, 15 mL	15-21.53 (1.58)	28.68 - 29.64	11.65 - 12.43	5.25 - 7.21	-	5.25 - 6.353 (2.17)	-	-	-	-	-	-	-	-	-	-	Manzoor et al. (2024)
Banana peel	Bread	10	-	12.6 (36.8)	34.5 (12.2)	10.7 (18.11)	-	2.7 (2.4)	-	38.9 (40.7)	-	-	-	-	-	-	-	-	Segura-Badilla et al. (2022)
	Biscuits	10, 20	-	6.2 - 5.3 (7.4)	2.3 - 13.1 (12.1)	30.2 -31.5 (28.3)	-	1.8 - 2.6 (1.5)	-	48.1 - 46.1 (50.7)	-	-	-	-	-	-	-	-	
Apple pomace, Indian gooseberry pomace, potato peels and bottle gourd peels	Bread	4, 5, 8, 10, 12, 15, 16, 20, 25	11.89 - 14.48 (2.23)	12.246 - 9.38 (12.2)	2.57 - 3.16 (10.79)	1.5 - 3.15 (1.74)	2.58 -4.67 (0.50)	6.26 - 8.28 (3.16)	255.2 - 365.46 (303.04)	54.32 - 70.36 (65.41)	-	-	-	-	-	-	-	-	Upadhyay et al. (2023)

Variation of colour analysis, rheological, physical, textural and sensorial proper

The addition of horticultural by-products to bakery products often results in a decrease in lightness (L*) values, likely due to Maillard and caramelization reactions, as well as the original colour of the HBP. Variations in redness (a*) and yellowness (b*) can also be attributed to the HBP colour (Djeghim et al., 2021; Upadhyay et al., 2023; Manzoor et al., 2024).

Rheological properties of bakery products can be measured or tested with different instruments (farinograph, alveograph, mixolab, visco anlyser), which provide data that can be correlated with final product's quality. Incorporating HBP into wheat-based doughs can modify their rheological properties, such as increased water absorption (WA) and dough development time (DDT) (Hussain et al., 2023). Changes in DDT and dough stability (DS) can be related to TDF composition of HBP.

HBP incorporation in wheat-based bakery products frequently leads to increased weight loss, specific volume, and moisture, along with decreased pH (Djeghim et al., 2021).

The texture of banana peel bagels shows higher hardness/firmness and cohesiveness, but lower gumminess and springiness (Manzoor et al., 2024).

Sensory scores for odour, flavour, taste, and texture generally decrease with higher HBP addition, though some wheat bread formulations show no impact or even improved scores for certain attributes.

Overall, the highest HBP addition without significant differences is often 5-10%, with a range of 2.5-25% reported in the literature.

Table 3. Influence of potential functional ingredients obtained from horticultural peels and skins on dough and final bakery product properties

HBP	Bakery product	% added	Dough rheological properties	Bakery products properties			Sensorial analysis	Reference
				Physical properties	Textural properties	Colour		
Pepper peels	Gluten-free bread	2.5, 5, 7.5	↓[1]K, [4]χ2 ↑[2]n, [3]r	↑[9]WL, [10]Vsp, moisture ↓pH	-	↓crust and crumb L*, crumb b* ↑crust and crumb a*, crumb b*	-	Djeghim et al. (2021)
Prickly pear peels	Gluten-free bread	2.5, 5, 7.5	↑[1]K, [4]χ2, [3]r ↓[2]n	↑[9]WL, [10]Vsp ↓pH, moisture	-	↓crust and crumb L*, crust b* ↑crust and crumb a*, crumb b*	-	
Prickly pear seed peels	Gluten-free bread	2.5, 5, 7.5	↑[1]K, [4]χ2 ↓[2]n, [3]r	↑[9]WL, [10]Vsp, pH and moisture	-	↓crust and crumb L* and b* ↑crust and crumb a*	-	
Apple pomace, Indian gooseberry pomace, potato peels and bottle gourd peels	Bread	4, 5, 8, 10, 12, 15, 16, 20, 25	-	-	-	↓L* ↑a*, b*	-	Upadhyay et al. (2023)
Banana peel	Bagels	10	-	-	↑hardness and cohesiveness ↓gumminess and springiness	↓L*, b* ↑a*	↑odour, taste and overall acceptability ↓crust colour and mastication	Manzoor et al. (2024)
Pumpkin peel	Biscuits	5, 10, 15	↑[5]WA, [6]DDT ↓[7]DS, [8]MTI, mixing time, peak height and spread factor	↓width ↑thickness	-	-	-	Hussain et al. (2023)
Banana peel	Bread	10	-	-	-	-	↓appearance, colour, texture	Segura-Badilla et al. (2022)
	Biscuits	10, 20	-	-	-	-	↓appearance, flavour, colour, smell and texture	

[1]K: consistency coefficient; [2]n: flow behaviour index; [3]r: statistical correlation coefficient; [4]χ2: chi-square; [5]WA: water absorption; [6]DDT: dough development time; [7]DS: dough stability; [8]MTI: mixing tolerance index; [9]WL: weight loss; [10]Vsp: specific volume;

The Influence of Fruit, Vegetable and Legumes Pomace on Bakery Products

The compositions of bakery products enhanced with potential functional components derived from various horticultural pomaces are summarized in Tables 4 and 5.

Variation of the total phenolic content, chemical composition and mineral content

The incorporation of horticultural by-product pomaces in bakery products, such as cakes, bread, and cookies, has been found to have various effects on their nutritional composition. Compared to control samples, cakes containing tomato, apple, and bread with apple and Indian gooseberry pomaces exhibited 40%, 52%, and 550% higher phenolic content, respectively (Bhat et al., 2022; Sudha et al., 2007; Upadhyay et al., 2023).

The addition of HBP pomaces to cakes and biscuits resulted in a decrease in protein content of up to 70% %. (Bhat et al., 2022; Jose et al., 2022; Ojeda et al., 2023; Sudha et al., 2007; Upadhyay et al., 2023). Oppositely, the inclusion of mushroom powder increased protein levels by 1 to 22% in comparison to control samples (Lu et al., 2018). Cookies enriched with pineapple pomace had higher protein content, while cakes with tomato and apple pomace presented lower protein levels. The TDF content increased significantly, ranging from 1.3 to 30 times, in bakery products formulated with HBP pomaces. Furthermore, higher ash and carbohydrate levels were observed in cookies with pineapple pomace and cakes with apple pomace (Jose et al., 2022; Sudha et al., 2007; Upadhyay et al., 2023). The effect on fat content varied, with cakes and bread containing tomato, apple, and Indian gooseberry pomaces exhibiting higher fat values compared to controls, while biscuits with pineapple pomace had lower fat content. However, none of the bakery products with fat content higher than 7 g kg^{-1} qualified for the "low fat" (<3 g fat kg^{-1} food product) and "fat-free" (<0.5 g fat kg^{-1} food product) nutrition claims (Regulation (EC) No. 1924/2006), except for the bread with apple and Indian gooseberry pomace, which could be classified as "low fat."

The reviewed studies did not report the influence of HBP pomace addition on the mineral content of the bakery products.

Table 4. Nutritional composition of bakery products with horticultural pomaces incorporated in bakery products

HBP	Bakery product	% added	TPC	Moisture	Protein	Fat	Ash	TDF	Energy value (Kcal/100 g)	Carbohydrate	Macrominerals			Microminerals					Reference
											Ca	Mg	Na	K	Fe	Mn	Zn	Cu	
Papaya seeds	Cupcakes	5, 10, 15, 20		20.09 - 21.56 (22.58)	13.40 - 18.55 (11.73)	20.69 - 21.07 (20.42)	3.09 - 4.65 (2.55)	1.44 - 4.22 (0.61)	389.51 - 399.15 (399.18)	-	-	-	-	-	-	-	-	-	Abdel-Hameed et al. (2023)
Pineapple pomace	Cookies with 400–251µm powder	5, 10, 15	-	3.26 - 3.49 (3.13)	11.88 - 11.19 (12.96)	18.50 - 15.33 (19.77)	1.70 - 1.76 (1.70)	2.09 - 2.22 (1.72)	-	62.45 - 66.20 (60.86)	-	-	-	-	-	-	-	-	Jose et al. (2022)
	Cookies with 250–150µm powder	5, 10, 15	-	3.12 - 3.40 (3.13)	12.09 - 11.59 (12.96)	18.37 - 16.23 (19.77)	1.72 - 1.75 (1.70)	2.02 - 2.32 (1.72)	-	62.57 - 64.57 (60.86)	-	-	-	-	-	-	-	-	
	Cookies with ≤ 149µm powder	5, 10, 15	-	3.29 - 3.58 (3.13)	11.22 - 10.94 (12.96)	18.35 - 16.32 (19.77)	1.72 - 1.75 (1.70)	2.23 - 2.45 (1.72)	-	63.68 - 65.04 (60.86)	-	-	-	-	-	-	-	-	
Shiitake mushroom	Sorghum biscuits	5, 10, 15	-	6.75 - 6.76 (5.66)	7.76 - 8.84 (7.21)	-	-	-	-	-	-	-	-	-	-	-	-	-	Lu et al. (2018)
Black ear mushroom	Sorghum biscuits	5, 10, 15	-	5.72 - 6.43 (5.66)	7.28 - 7.38 (7.21)	-	-	-	-	-	-	-	-	-	-	-	-	-	
Silver ear mushroom	Sorghum biscuits	5, 10, 15	-	5.71 - 6.24 (5.66)	7.20 - 7.33 (7.21)	-	-	-	-	-	-	-	-	-	-	-	-	-	
Vinal (Neltuma ruscifolia) residue	Bread	5	-	8.90	10.20	2.40	4.50	21.20	-	53.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Apple pomace	Cake	25	3.15 (2.07)	21.8 (20.9)	8.46 (8.5)	20.5 (19.3)		14.20 (0.47)	-	-	-	-	-	-	-	-	-	-	Sudha et al. (2007)
Apple and Indian gooseberry pomace, potato and bottle gourd peels	Bread	4, 5, 8, 10, 12, 15, 16, 20, 25	11.89 - 14.48 (2.23)	12.246 - 9.38 (12.2)	2.57 -3.16 (10.79)	1.5 - 3.15 (1.74)	2.58 - 4.67 (0.50)	6.26 - 8.28 (3.16)	255.2 - 365.46 (303.04)	54.32 - 70.36 (65.41)		-	-	-	-	-	-	-	Upadhyay et al. (2023)

Variation of colour analysis, rheological, physical, textural and sensorial properties

A darker colour is commonly observed in bakery products formulated with HBP pomaces, while cookies with pineapple pomace and silver ear mushroom had a higher L* value. This can be attributed to Maillard and caramelization reactions, as well as the original colour of the HBP. Differences in a* and b* values were likely related to the original HBP colour (Bhat et al., 2022; Djeghim et al., 2021; Din et al., 2024; Upadhyay et al., 2023).

Dough rheology changes in enriched bakery products are shown in Table 5. Biscuits enriched with pumpkin pomace had increased WA and DDT, but decreased dough stability (DS). The opposite was true for light-grain rye semi-finished products enriched with apple and pumpkin pomace, where WA decreased (Hussain et al., 2023; Sadygova et al., 2021). Changes in dough WA can be linked to protein content, dietary fiber structure, molecular interactions, particle size, and fiber porosity. The impact of HBP pomaces on starch pasting properties, such as peak viscosity (PV), though viscosity (TV), final viscosity (FV), setback viscosity (SV) and breakdown viscosity (BV), as well as pasting time and temperature, also varied in cookies with pineapple pomace and sorghum biscuits with mushrooms. These properties increased in products enriched with pumpkin pomace and decreased with shiitake mushroom addition (Jose et al., 2022; Lu et al., 2018).

Physical characteristics, such as volume and specific volume (Vsp), increased for enriched gluten-free bread with apple and orange pomace, except for tomato pomace and cake with apple pomace (Djeghim et al., 2021; Sudha et al., 2007). Other physical properties, like weight, frequently increased, while thickness and diameter decreased (Jose et al., 2022; Lu et al., 2018; Hussain et al., 2023).

As shown in Table 5, a consistent increase was observed for hardness/firmness, fracturability, chewiness, and gumminess, with a decrease in cohesiveness (Jose et al., 2022; Upadhyay et al., 2023; Mildner-Szkudlar et al., 2016; Lu et al., 2018).

Regarding sensory analysis, scores for flavour, taste, texture, and overall acceptability usually decreased with increasing addition of HBP pomaces to bakery formulations. However, some formulations showed better scores for these characteristics (Bhat et al., 2022; Sudha et al., 2007; Ojeda et al., 2023; Upadhyay et al., 2023).

Based on the analysis of various parameters, especially sensory analysis, the highest incorporation of HBP pomaces in bakery products like cakes, biscuits, muffins, and bread without significant overall differences was often 5 and 10%, with the maximum addition at 30% and minimum at 2.5%.

Table 5. Influence of potential functional ingredients obtained from horticultural pomaces on dough and final bakery product properties

HBP	Bakery product	% added	Dough rheological properties	Bakery products properties			Sensorial analysis	Reference
				Physical properties	Textural properties	Colour		
Tomato pomace	Cakes	2, 4	-	-	-	↑crust L*, crust and crumb a*, crumb b* ↓crumb L*, crust b*	↑appearance and texture ↓flavour, Aftertaste and overall acceptability	Bhat et al. (2022)
Orange pomace	Gluten-free bread	2.5, 5, 7.5	↓[1]K, [2]χ2, [3]n ↑[4]r	↑[20]WL, [21]Vsp ↓pH, moisture	-	↓crust and crumb L* ↑crust and crumb a* and b*	-	Djeghim et al. (2021)
Apple pomace	Gluten-free bread	2.5, 5, 7.5	↑[2]χ2 ↓[1]K, [3]n, [4]r	↑[20]WL, [21]Vsp, moisture ↓pH	-	↓crust and crumb L* and b* ↑crust and crumb a*	-	
Tomato pomace	Gluten-free bread	2.5, 5, 7.5	↓[1]K, [2]χ2 ↑[3]n, [4]r	↓[20]WL, [21]Vsp ↑pH, moisture	-	↑crust and crumb L* and b*, crumb a* ↓crust a*	-	
Pineapple pomace	Cookies with 400–251µm powder	5, 10, 15	↓[5]PV, [6]TV, [7]BV, [8]FV, [9]SV, [10]WSI, [11]SP ↑[12]WA, [13]OA, [14]PT and pasting time	↓thickness and diameter ↑aw, spread ratio and weight	↓hardness and fracturability	↑L*, b* ↓a*	-	Jose et al. (2022)
	Cookies with 250–150µm powder	5, 10, 15	↓[5]PV, [6]TV, [7]BV, [8]FV, [9]SV, [10]WSI, [11]SP ↑[12]WA, [13]OA, [14]PT and pasting time	↓spread ratio and diameter ↑aw, thickness, weight	↓hardness ↑fracturability	↑L* ↓a*, b*	-	
	Cookies with ≤ 149µm powder	5, 10, 15	↓[5]PV, [6]TV, [7]BV, [8]FV, [9]SV, [10]WSI, [11]SP ↑[12]WA, [13]OA, [14]PT and pasting time	↓thickness, diameter and weight ↑aw and spread ratio	↓hardness ↑fracturability	↓L* ↑a*, b*	-	
Apple and Indian gooseberry pomace, potato peels and bottle gourd peels	Bread	4, 5, 8, 10, 12, 15, 16, 20, 25	↑[11]SP, [15]EC, [16]FC	↑weight ↓volume and [14]Vsp	↑hardness ↓cohesiveness	↓L* ↑a*, b*	↑colour, texture, flavour, appearance and overall acceptability	Upadhyay et al. (2023)
Apple pomace	Cake	10, 20, 30	-	↓volume and weight ↑ρ and texture	-	-	↓crust and crumb colour, crumb grain, crumb texture, eating quality and overall acceptability	Sudha et al. (2007)
Raspberry pomace	Muffins baked at 140 °C/30min	10, 20	-	-	↑hardness and cohesiveness ↓gumminess and chewiness	-	-	Mildner-Szkudlarz et al. (2016)
	Muffins baked at 180 °C/20min	10, 20	-	-	↑hardness, cohesiveness, gumminess and chewiness	-	-	
	Muffins baked at 240 °C/15min	10, 20	-	-	↑hardness, cohesiveness, gumminess and chewiness	-	-	
Cranberry pomace	Muffins baked at 140 °C/30min	10, 20	-	-	↑hardness, gumminess and chewiness ↓cohesiveness	-	-	
	Muffins baked at 180 °C/20min	10, 20	-	-	↑hardness, gumminess and chewiness ↓cohesiveness	-	-	
	Muffins baked at 240 °C/15min	10, 20	-	-	↑hardness, gumminess and chewiness ↓cohesiveness	-	-	
Apple pomace	Light-grain rye semi-finished product	15	↓[12]WA, kneading and gluten ↑viscosity, amylase and retrogradation	-	-	-	-	Sadygova et al. (2021)
Pumpkin pomace	Light-grain rye semi-finished product	15	↓[12]WA, kneading and gluten ↑viscosity, amylase and retrogradation	-	-	-	-	
Shiitake mushroom	Sorghum biscuits	5, 10, 15	↑[5]PV and [7]BV ↓[6]TV, [8]FV, [9]SV and [14]PT	↓diameter, thickness and [13]WL ↑weight	↑hardness	↓L*, b*, chroma, hue-angle ↑a*	-	Lu et al. (2018)
Black ear mushroom	Sorghum biscuits	5, 10, 15	↑[5]PV, [6]TV, [7]BV, [8]FV and [9]SV ↓[14]PT	↓diameter, thickness and [13]WL ↑weight	↑hardness	↓L*, a*, b*, chroma, hue-angle	-	
Silver ear mushroom	Sorghum biscuits	5, 10, 15	↑[5]PV, [6]TV, [7]BV, [8]FV and [9]SV ↓[14]PT	↓diameter and thickness ↑weight and [13]WL	↑hardness	↑L*, a*, b*, chroma, hue-angle	-	
Vinal (Neltuma ruscifolia) residue	Bread	5	-	-	-	-	↑colour, odour, texture and overall acceptability	Ojeda et al. (2023)
Pumpkin pomace	Biscuits	5, 10, 15	↑[17]WA, [17]DDT ↓[18]DS, [19]MTI, mixing time, peak height and spread factor	↓width ↑thickness	-	-	-	Hussain et al. (2023)

[1]K: consistency coefficient; [2]χ2: chi-square; [3]n: flow behaviour index; [4]r: statistical correlation coefficient; [5]PV: peak viscosity; [6]TV: trough viscosity; [7]FV: final viscosity; [8]BV: breakdown viscosity; [9]SV: setback viscosity; [10]WSI: water solubility index; [11]SP: swelling power; [12]WA: water absorption; [13]OA: oil absorption; [14]PT: pasting temperature; [15]EC: emulsion capacity; [16]FC: foaming capacity; [17]DDT: dough development time; [18]DS: dough stability; [19]MTI: mixing tolerance index; [20]WL: weight loss; [21]Vsp: specific volume.

The Influence of Fruit, Vegetable and Legumes Seeds on Bakery Products

The nutritional profile of bakery goods fortified with potentially functional ingredients derived from seeds is summarized in Tables 6 and 7.

Variation of the total phenolic content, chemical composition and mineral content

Higher phenolic content values, compared to the control samples, were found in cookies and pita bread containing pumpkin seeds had 11% and 6%, respectively. However, pan bread with yellow pumpkin seeds recorded a 10% decrease in phenolic content (Weldeyohanis Gebremariam et al., 2024; Aljahani, 2022).

Compared to the control samples, protein content increased between 7% and 98% in formulations with papaya seeds, pumpkin seeds, amaranth seeds, and vinal seeds. A small 0.3% protein reduction was reported for pita bread with yellow pumpkin seed addition. Overall, pumpkin seeds and vinal seeds had higher protein content, while amaranth seeds had lower protein content (Abdel-Hameed et

al., 2023; Weldeyohanis Gebremariam et al., 2024; Aljahani, 2022; Apostol et al., 2020; Bojňanská et al., 2024; Matseychik et al., 2021; Ojeda et al., 2023).

The incorporation of HBP seeds increased the TDF content from 159% to 591% in all bakery products. Compared to the control samples, the addition of seeds raised the fat concentration from 1% to 12%, while pomegranate peel reduced the fat concentration with 3-153%. The fat content can be used to determine eligibility for the "low fat" and "fat-free" nutrition claims (Regulation (EC) No. 1924/2006). Therefore, the pita bread with yellow pumpkin seeds qualifies for the "fat-free" nutrition claim, while the breads with pumpkin seeds and vinal endocarp are "low fat" (Abdel-Hameed et al., 2023; Weldeyohanis Gebremariam et al., 2024; Aljahani, 2022; Apostol et al., 2020).

Cookies and bread enriched with pumpkin seeds reported increased mineral content compared to the control samples (Weldeyohanis Gebremariam et al., 2024; Apostol et al., 2020).

Table 6. Nutritional composition of bakery products with horticultural seeds incorporated in bakery products

HBP	Bakery product	% added	TPC	Moisture	Protein	Fat	Ash	TDF	Energy value (Kcal/100 g)	Carbohydrate	Macrominerals			Microminerals					Reference
											Ca	Mg	Na	K	Fe	Mn	Zn	Cu	
Papaya seeds	Cupcakes	5, 10, 15, 20		20.09 - 21.56 (22.58)	13.40 - 18.55 (11.73)	20.69 - 21.07 (20.42)	3.09 - 4.65 (2.55)	1.44 - 4.22 (0.61)	389.51 - 399.15 (399.18)	-	-	-	-	-	-	-	-	-	Abdel-Hameed et al. (2023)
Pumpkin seeds	Cookies	6, 10.5, 15	43.19 (38.72)	2.52 (3.05)	14.28 (11.49)	17.73 (13.99)	2.16 (1.77)	2.41 (0.93)	469.81 (452.00)	-	46.25 (32.23)	51.33 (40.12)	-	-	3.20 (2.12)	-	2.63 (1.42)	-	Weldeyohanis Gebremariam et al. (2024)
Yellow pumpkin seeds	Pan bread	5, 10, 15	5.21 - 5.37 (5.95)	8.04 - 8.27 (8.42)	11.15 - 11.41 (10.60)	2.44 - 3.12 (2.17)	2.89 - 3.48 (2.72)	-	-	75.14 - 74.21 (76.08)	-	-	-	-	-	-	-	-	Aljahani (2022)
	Pita bread		5.21 - 5.30 (5.01)	7.69 - 9.51 (8.25)	10.33 - 10.81 (10.84)	0.20 - 0.27 (0.18)	1.91 - 2.41 (1.68)	-	-	77.86 - 79.29 (79.05)	-	-	-	-	-	-	-	-	
Pumpkin seeds	Wheat flour and dough	5, 10, 15	-	-	15.35 - 18.65 (13.70)	1.66 - 2.79 (1.10)	0.90 - 1.59 (0.55)	3.18 - 5.74 (1.90)	-	82.09 - 76.97 (84.65)	58.41 - 87.83 (43.70)	69.77 - 113.9 (47.70)	28.22 - 29.81 (30.60)	239.4 - 344.2 (187)	4.42 - 11.05 (1.10)	1.27 - 2.68 (0.56)	5.65 - 6.03 (5.45)	0.85 - 0.98 (0.78)	Apostol et al. (2020)
Amaranth seeds	Bread	5, 10, 15	-	11.8 - 11.9 (11.9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Bojňanská et al. (2024)
Amaranth seeds	Rolls	-	-	40.3 - 36.4 (36.0)	13.2 - 12.1 (6.1)	-	3.04 - 2.6 (1.5)	-	119.0 - 118.0 (140.0)	-	-	-	-	-	-	-	-	-	Matseychik et al. (2021)
Vinal (Neltuma ruscifolia) seeds	Bread	5	-	5.90	30.60	4.60	3.20	20.90	-	35.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Vinal (Neltuma ruscifolia) endocarp	Bread	5	-	8.90	11.80	2.10	3.90	38.60	-	35.00	-	-	-	-	-	-	-	-	

Variation of colour analysis, rheological, physical, textural and sensorial properties

A decrease in crust and crumb L* value is typically observed in bread and cupcake formulations containing pumpkin and papaya seeds, respectively. This can be attributed to Maillard and caramelization reactions, as well as the original colour of the horticultural by-products, leading to a darker appearance and differences in a* and b* values (Abdel-Hameed et al., 2023; Aljahani, 2022).

The effects of HBP seeds on dough rheology in enriched bakery products are summarized in Table 7. Water absorption increases with the addition of HBP seeds, except for dough with pumpkin seeds. The influence of HBP seeds on dough development time, dough stability, and mixing tolerance index varies depending on the type and concentration of seeds used, but generally increases (Bojňanská et al., 2024; Hussain et al., 2023; Apostol et al., 2020).

Physical characteristics such as volume, specific volume, thickness, and diameter are affected by the addition of HBP seeds. These changes can be related to the gluten and TDF content and structure (Matseychik et al., 2021; Abdel-Hameed et al., 2023; Aljahani, 2022; Hussain et al., 2023).

Scarce data is available on textural parameters, but an increase in cohesiveness and springiness, as well as a decrease in hardness, adhesiveness, and gumminess, have been reported with the addition of yellow pumpkin seeds to pan and pita bread (Aljahani, 2022).

Sensory analysis often shows that the addition of HBP seeds to bakery formulations decreases scores related to aroma, flavour, taste, colour, and texture. However, in the bread formulation with vinal endocarp addition, there is no significant difference or a slight increase in the scores for these sensory attributes (Abdel-Hameed et al., 2023; Aljahani, 2022; Ojeda et al., 2023).

Based on the information gathered from all the analyzed parameters, the highest incorporation of HBP seeds in bakery products without significant overall differences is frequently 5%. The maximum addition value possible is 20%, while the minimum is 5%.

Table 7. Influence of potential functional ingredients obtained from horticultural seeds on dough and final bakery product properties

HBP	Bakery product	% added	Dough rheological properties	Bakery products properties			Sensorial analysis	Reference
				Physical properties	Textural properties	Colour		
Amaranth seeds	Rolls	5, 10, 15	-	↑humidity, porosity, acidity	-	-	-	Matseychik et al. (2021)
Amaranth seeds	Bread	5, 10, 15	↑[1]WA, [2]DDT, [3]DS ↓consistency	-	-	-	-	Bojňanská et al. (2024)
Papaya seeds	Cupcakes	5, 10, 15, 20	-	↑height and weight before and after baking, volume and [4]Vsp	-	↓crust and crumb L*, b* and chroma, crumb a* ↑crust a*, crust and crumb ΔE and hue angle	↓aroma, flavour, Aftertaste and overall acceptability	Abdel-Hameed et al. (2023)
Yellow pumpkin seeds	Flour and dough	5, 10, 15	↓[5]PV, [6]BV, [7]FV, [8]SV and [9]PT	-	-	-	-	Aljahani (2022)
	Pan bread	5, 10, 15	-	↑weight and aw ↓volume and [4]Vsp	↓hardness, adhesiveness and gumminess ↑cohesiveness and springiness	↓crust and crumb L* ↑crust and crumb a* and b*	↓crust colour, symmetry, break and shred, crumb colour, grain and texture, flavour and overall acceptability	
	Pita bread		-	↑weight and aw ↓volume and [4]Vsp	↓hardness, adhesiveness and gumminess ↑cohesiveness and springiness	↓crust L* ↑crumb L*, crust and crumb a* and b*	↓crust colour, symmetry, break and shred, crumb colour, grain and texture, flavour and overall acceptability	
Pumpkin seeds	Biscuits	5, 10, 15	↑[1]WA, [2]DDT ↓[3]DS, [10]MTI, mixing time, peak height and spread factor	↓width ↑thickness	-	-	-	Hussain et al. (2023)
Pumpkin seeds	Wheat flour and dough	5, 10, 15	↓[1]WA ↑[3]DS and amplitude	-	-	-	-	Apostol et al. (2020)
Vinal (Neltuma ruscifolia) seeds	Bread	5	-	-	-	-	↓colour, odour, texture and overall acceptability	Ojeda et al. (2023)
Vinal (Neltuma ruscifolia) endocarp	Bread	5	-	-	-	-	↑colour, odour, texture and overall acceptability	

[1]WA: water absorption; [2]DDT: dough development time; [3]DS: dough stability; [4]Vsp: specific volume; [5]PV: peak viscosity; [6]BV: breakdown viscosity; [7]FV: final viscosity; [8]SV: setback viscosity; [9]PT: pasting temperature; [10]MTI: mixing tolerance index.

The Influence of Petals, Leaves, Stems and Other Floral By-Products on Bakery Products

The composition of bakery products enriched with ingredients from petals, stems, leaves and other floral by-products is summarized in Tables 8 and 9.

Variation of the total phenolic content, chemical composition and mineral content

Higher phenolic content is generally observed in baked goods like cakes, cookies, bread, and muffins that are enriched with by-products from saffron, broccoli, tea, and cauliflower. Compared to control samples, these values increased significantly, from 4% to 197% (Bhat et al., 2022; Bhat et al., 2018; Cerda-Bernad & Frutos, 2023; Drabińska et al., 2018; Koh et al., 2023; Tukassar et al., 2023).

Bhat et al. (2022) also reported a non-significant decrease ($p>0.05$) in TPC of saffron and tomato enriched cakes during a storage period of 0-15 days, possibly due to the by-products' high antioxidant activity. Baking significantly increased TPC of cookies supplemented with saffron stigmas due to the release of bound phenolics and the formation of Malanoidins produced in the Maillard reaction, though a gradual decline was seen over storage, becoming significant after 9 months (Bhat et al., 2018).

In contrast, Drabińska et al. (2018) found no significant impact of thermal processing on TPC or antioxidant activity in gluten-free cakes with broccoli leaves. Similarly, Tukassar et al. (2023) reported that baking caused less nutrient loss than prolonged mixing in cauliflower-enriched muffins and improved phenolic bioavailability by releasing cell wall-bound phenolic compounds.

Protein content also increased, from 1% to 31%, in formulations containing by-products from saffron, tea, banana blossoms, and cauliflower. However, a slight reduction of 0.3% was reported for bread with saffron tepals, and little to no increase was seen in patties with spinach leaves and cookies with tea leaves. Overall, higher protein was found in cakes with banana blossoms, muffins with cauliflower, and bread with vinal pods, while saffron stigmas and tea leaves had lower protein content (Bhat et al., 2022; Bhat et al., 2018; Koh et al., 2023; Ojeda et al., 2023; Sapozhnikov et al., 2019; Tasnim et al., 2020; Tukassar et al., 2023).

The TDF content increased greatly, from 25% to 412%, in baked goods enriched with tea leaves, banana blossoms, and cauliflower by-products (Koh et al., 2023; Tasnim et al., 2020; Tukassar et al., 2023). The incorporation of floral by-products slightly raised fat

concentration, from 0.3% to 3%, for saffron, tea, spinach, and banana, while cauliflower increased it by 52%. Conversely, these by-products generally lowered carbohydrate content, from 6% to 20% (Bhat et al., 2022; Bhat et al., 2018; Koh et al., 2023; Ojeda et al., 2023; Sapozhnikov et al., 2019; Tasnim et al., 2020; Tukassar et al., 2023).

Bread with saffron tepals and muffins with cauliflower reported increased mineral content compared to controls. Cake with banana blossoms also had 11% and 122% increases in calcium and iron, respectively (Cerda-Bernad & Frutos, 2023; Tasnim et al., 2020; Tukassar et al., 2023).

Table 8. Nutritional composition of bakery products with horticultural petals, leaves, stems and other floral by-products incorporated in bakery products

HBP	Bakery product	% added	TPC	Moisture	Protein	Fat	Ash	TDF	Energy value (Kcal/100 g)	Carbohydrate	Macrominerals			Microminerals					Reference
											Ca	Mg	Na	1K	Fe	Mn	Zn	Cu	
Saffron stigmas	Cakes	0.05, 0.1	0.66 - 0.75 (0.62)	30.8 - 36.1 (33.4)	6.79 - 6.68 (6.57)	30.15 - 30.21 (30.11)	1.76 - 1.80 (1.77)	-	-	-	-	-	-	-	-	-	-	-	Bhat et al. (2022)
Saffron stigmas	Cookies	0.05, 0.1	0.86 - 0.94 (0.90)	2.29 - 2.37 (2.43)	6.14 - 6.11 (6.13)	27.13 - 27.41 (27.18)	1.91 - 1.92 (1.83)	-	-	-	-	-	-	-	-	-	-	-	Bhat et al. (2018)
Saffron tepals	Wheat bread	2.5, 5, 10	111 - 272 (91.31)	25.47 - 32.44 (31.12)	-	-	2.95 - 3.79 (2.75)	-	-	-	56.18 - 91.63 (39.22)	34.72 - 41.61 (31.21)	713 - 605 (641)	196 - 277 (162)	6.01 - 15.87 (2.01)	1.22 - 1.42 (1.03)	0.97 - 1.03 (0.77)	-	Cerda-Bernad & Frutos (2023)
	Spelt bread	2.5, 5, 10	124 - 261 (102)	29.51-31.89 (32.71(	-	-	3.37 - 3.95 (3.05)	-	-	-	52.74 - 94.56 (33.47)	34.46 - 48.46 (33.95)	713 - 743 (810)	178 - 289 (153)	6.27 - 17.85 (3.27)	0.91 - 1.37 (0.74)	0.78 - 0.96 (0.71)	-	
Broccoli leaves	Gluten-free mini sponge cakes	2.5, 5, 7.5	0.77 - 0.99 (0.46)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Drabińska et al. (2018)
Green tea leaves	Gluten free shortbread cookie	8	80 (30)	2.18 (1.62)	6.88 (6.86)	20.60 (20.17)	2.04 (1.43)	14.47 (7.70)	428.21 (457.84)	53.83 (62.21)	-	-	-	-	-	-	-	-	Koh et al. (2023)
Oolong tea leaves	Gluten free shortbread cookie	8	60 (30)	2.35 (1.62)	6.89 (6.86)	20.80 (20.17)	1.91 (1.43)	14.89 (7.70)	427.36 (457.84)	53.16 (62.21)	-	-	-	-	-	-	-	-	
Black tea leaves	Gluten free shortbread cookie	8	65 (30)	2.19 (1.62)	6.84 (6.86)	20.39 (20.17)	1.96 (1.43)	14.75 (7.70)	426.37 (457.84)	53.87 (62.21)	-	-	-	-	-	-	-	-	
Vinal (Neltuma ruscifolia) pods	Bread	5	-	8.70	12.80	2.40	4.10	31.10		41.00	-	-	-	-	-	-	-	-	Ojeda et al. (2023)
Spinach leaves	Wheat flour roll	4	-	-	6.0 (6.0)	6.2 (6.5)	1.7 (0.97)	-	138 (144)	20.9 (22.3)	-	-	-	-	-	-	-	-	Sapozhnikov et al. (2019)
	Curd patty	4	-	-	12.8 (12.1)	10.0 (9.8)	0.91 (0.75)	-	271 (262)	38.1 (36.8)	-	-	-	-	-	-	-	-	
	Lingonberry patty	4	-	-	6.0 (5.9)	9.2 (9.1)	0.97 (0.63)	-	289 (285)	49.0 (48.7)	-	-	-	-	-	-	-	-	
Banana blossoms	Cake	4, 6, 8	-	16.78 - 20.31 (14.32)	9.10 - 11.20 (8.53)	22.70 - 23.95 (22.10)	1.46 - 1.89 (1.23)	3.10 - 3.45 (2.75)	440.54 - 430.99 (448.30)	49.96 - 42.66 (53.82)	160.2 - 168.5 (151.5)	-	-	-	21.50 - 33.30 (15.0)	-	-	-	Tasnim et al. (2020)
Cauliflower stems, stalks and leaves	Muffins	10, 20, 30	282.40 - 561.20 (193.10)	26.13 - 23.91 (27.26)	8.93 - 9.70 (7.53)	6.55 - 6.82 (6.44)	2.01 - 2.38 (1.83)	1.17 - 3.74 (0.73)	126 - 106 (134)	55.23 - 53.46 (56.24)	189.69 - 244.56 (171.47)	53.94 - 61.19 (51.35)	119.51 - 167.76 (108.19)	-	2.66 - 6.56 (1.66)	-	1.29 - 1.58 (1.19)	0.68 - 1.99 (0.26)	Tukassar et al. (2023)

Variation of colour analysis, rheological, physical, textural and sensorial properties

Crust and crumb L* generally decreased with the addition of floral by-products. This is due to Maillard and caramelization reactions, as well as the original colour of the horticultural by-products. The original colour of the by-product also explains the differences in a* and b* values (Bhat et al., 2022; Bhat et al., 2018; Cerda-Bernad & Frutos, 2023; Koh et al., 2023; Tasnim et al., 2020; Tukassar et al., 2023).

Data on dough rheology changes in bakery products enriched with floral by-products is limited. The pasting properties PV, BV, FV and SV increased with the addition of cauliflower stems, stalks, and leaves to muffins compared to control samples, while trough viscosity, peak time, and pasting temperature decreased (Tukassar et al., 2023).

Physical characteristics like Vsp, spread factor, density, thickness, and diameter are affected by the addition of floral by-products. Changes in these parameters can be attributed to gluten, TDF content and the structure of the respective HBP (Bhat et al., 2018; Rizi et al., 2024; Chaalal et al., 2023; Koh et al., 2023; Tasnim et al., 2020; Tukassar et al., 2023).

Textural data shows an increase in hardness, firmness, gumminess, and chewiness, while cohesiveness and springiness generally decrease with the addition of floral by-products (Bhat et al., 2018; Cerda-Bernad & Frutos, 2023; Rizi et al., 2024; Koh et al., 2023; Tasnim et al., 2020; Tukassar et al., 2023).

Sensory analysis indicates that the addition of floral by-products such as leaves, petals, stems, and stalks to bakery formulations usually decreases scores for aroma, flavour, taste, and texture. However, in many formulations, the scores for colour, odour, appearance, and even aroma, taste, and texture show a significant increase compared to control samples (Bhat et al., 2022; Bhat et al., 2018; Cerda-Bernad & Frutos, 2023; Rizi et al., 2024; Chaalal et al., 2023; Koh et al., 2023; Tasnim et al., 2020; Tukassar et al., 2023; Ojeda et al., 2023; Sapozhnikov et al., 2019). These scores suggest that bakery formulations with floral by-products are generally better accepted by consumers than those with peels, skins, pomaces, or seeds.

Based on the analysis of various parameters, especially sensory analysis, the highest incorporation of floral by-products in bakery formulations without significant overall differences is frequently 4-5%. The maximum addition value possible is 30%, while the minimum is 0.05%.

Table 9. Influence of potential functional ingredients obtained from horticultural petals, leaves, stems and other floral by-products on dough and final bakery product properties

HBP	Bakery products	% added	Dough rheological properties	Bakery products properties			Sensorial analysis	References
				Physical properties	Textural properties	Colour		
Saffron stigmas	Cakes	0.05, 0.1	-	-	-	↓crust and crumb L* ↑crust and crumb a* and b*	↑appearance and texture ↓flavour, Aftertaste and overall acceptability	Bhat et al. (2022)
Saffron stigmas	Cookies	0.05, 0.1	-	↓weight, diameter and spread ratio ↑thickness	↓hardness	↑L*, b* ↓a*	↓texture ↑appearance, mouth feel, flavour and overall acceptability	Bhat et al. (2018)
Saffron tepals	Wheat bread	2.5, 5, 10	-	-	↑hardness, gumminess and chewiness ↓cohesiveness and springiness	↓crust and crumb L* and a*, crust b* ↑crumb b*	↑evenness colour of crust, sweetness, flower-ID, floral-herbaceous taste, sourness astringency, bitterness, aftertaste, hardness, cohesiveness, adhesiveness, stickiness ↓crispiness, saltiness	Cerda-Bernad & Frutos (2023)
	Spelt bread	2.5, 5, 10	-	-	↑hardness, gumminess and chewiness ↓cohesiveness and springiness	↓crust and crumb L*, crust a* and b* ↑crumb a* and b*	-	
Saffron petals	Mung bean sourdough bread	10	-	↑porosity ↓pH	↓hardness, gumminess ↑resilience and cohesiveness	-	↑aroma and taste ↓chew-ability, colour, mouth feel and overall acceptability	Rizi et al. (2024)
Saffron stigmas	Biscuits	0.1	-	↓humidity, pH ↑acidity			↑colour, odour, taste, texture, aspect and overall acceptability	Chaalal et al. (2023)
Green tea leaves	Gluten free shortbread cookie	8	-	↓ρ, pH ↑spread factor, loss rate and aw	↑hardness	↓L*, a*, b*	↑appearance, taste, smell, texture and overall acceptability	Koh et al. (2023)
Oolong tea leaves	Gluten free shortbread cookie	8	-	↓ρ, pH ↑spread factor, loss rate and aw	↑hardness	↓L*, a*, b*	↓appearance, taste, smell, texture and overall acceptability	
Black tea leaves	Gluten free shortbread cookie	8	-	↓ρ, pH ↑spread factor, loss rate and aw	↑hardness	↓L*, a*, b*	↓appearance, taste, smell, texture and overall acceptability	
Banana blossoms	Cake	4, 6, 8	↑[1]WA, [2]OA, [3]FC ↓[4]SP	↓ρ ↑solubility	↑hardness, gumminess and resilience ↓cohesiveness, springiness and chewiness	↓L*, b* ↑a*	↓colour, flavour, texture and overall acceptability	Tasnim et al. (2020)
Cauliflower stems, stalks and leaves	Muffins	10, 20, 30	↑[6]PV, [7]BV, [8]FV, [9]SV ↓[10]TV, peak time and [11]PT	↓[5]Vsp	↑hardness and chewiness ↓cohesiveness and springiness	↓L*, b* ↑a*, ΔE*	↓appearance, taste, aroma, texture, softness, palatability, colour and overall acceptability	Tukassar et al. (2023)
Vinal (Neltuma ruscifolia) pods	Bread	5	-	-	-	-	↑colour, odour, texture and overall acceptability	Ojeda et al. (2023)
Spinach leaves	Wheat flour roll	4	-	-	-	-	↓scent and taste ↑consistency	Sapozhnikov et al. (2019)
	Curd patty	4	-	-	-	-	↓scent ↑taste and colour	
	Lingonberry patty	4	-	-	-	-	↑appearance, colour and consistency ↓taste	

[1]WA: water absorption; [2]OA: oil absorption; [3]FC: foaming capacity; [4]SP: swelling power; [5]Vsp: specific volume; [6]PV: peak viscosity; [7]BV: breakdown viscosity; [8]FV: final viscosity; [9]SV: setback viscosity; [10]TV: trough viscosity; [11]PT: pasting temperature

CONCLUSIONS

The exploration of horticultural by-products as valuable sources of functional ingredients in bakery products has been extensively researched. Fruit and vegetable by-products, which constitute a significant portion of food waste, are studied for their potential to enhance baked goods quality. By-products are commonly processed into powders or flours,

which are easily integrated into bakery formulations.

Research shows increasing inclusion of these powders correlates positively with higher phenolic content and antioxidant capacity in bakery products. These bioactive compounds have potential health benefits.

Understanding sensory attributes and ensuring safety are crucial for consumer acceptance and market success. Comprehensive investigations into their phenolic profile, chemical and mineral content, as well as sensory, textural and rheological properties will provide insights to optimize formulations, meet consumer preferences, ensure product safety, and leverage the potential of horticultural by-products for human health and sustainable food systems.

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RESEARCH ON THE APPEARANCE AND EVOLUTION OF *MONARTHROPALPUS BUXI* (LABOULBÈNE) IN THE BOXWOOD PLANTATIONS OF IASI COUNTY

Iuliana Elena GOLACHE[1], Iulia MINEAȚĂ[1], Ionel PERJU[1], Ionuț Vasile UNGUREANU[1], Monica HEREA[2], Mihai TĂLMACIU[2]

[1]Research Station for Fruit Growing Iași, 3 Ion Voda cel Viteaz, Miroslava, Iași, Romania
[2]"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: iulia_mineata@yahoo.com

Abstract

Boxwood species, valued for their ecological adaptability, face growing threats from pests, notably the boxwood leafminer (Monarthropalpus buxi Laboulbène), which has severely impacted landscapes and nurseries in recent years. The aim of this study was to monitoring the evolution and ecology of Monarthropalpus buxi according to the local biotope and testing two insecticides in order to control the pest in North-Eastern part of Romania. The life cycle of boxwood leafminer was described in relation to GDD. The appearance of the first adults in 2022 was recorded at an accumulation of 196.2 GDD on 09th May, and the appearance of adults began at an accumulation of 191.7 GDD on 02nd May in 2023. The maximum flight curve was recorded at accumulations between 262.7 GDD (2022), respectively 246.3 GDD (2023). Last appearance of adults was observed on 28th May (2022) and 25 Mayth (2023). The larval stages in 2022 developed at accumulations between 675.7 - 1298.3 GDD and in the following year the larvae developed between 670.4 - 1317.1 GDD. Statistical methods were used to interpret the obtained results.

Key words*: buxus, boxwood leafminer, Growing Degree - Days, monitoring, temperature.*

INTRODUCTION

Boxwood (*Buxus* spp.) are valued in landscape design for the characteristics of its foliage and versatility. They are used in landscaping in a range of sizes and shapes, from low-growing varieties suitable for hedges and edging to taller specimens that can be used as focalized points or izolated plants. This versatility allows them to be used in various ways to create different effects in the landscape. The permanent interest in this plant has increased the production of boxwood in nurseries all over the world (Weiland et al., 2022).

Ever since the last century, the cultivation and production of boxwood has been challenged by the attack of several harmful arthropods and the threat of pathogens with negative effects on the general appearance of the plant (Brewer et al., 1980; Baylac & Daufresne, 1996; Ivors et al., 2012; Dhakal et al., 2022).

Boxwood leafminer, known scientifically as *Monarthropalpus buxi* or *Monarthropalpus flavus* (Diptera: Cecidomyiidae), is a serious key pest of boxwood, both in the landscape and especially in nurseries (d`Eustachio & Raupp, 2001). This monophagous small fly is native to Europe, but widespread in all buxus cultivation areas distributed including in United States of America. In Romania, *Monarthropalpus buxi* was described by Perju et al. (2001) and it was reported in the urban areas of Cluj County and in the parks of Bucharest (Soporan et al., 2012; Soporan et al., 2015; Ciceoi et al., 2017).

The larvae of this pest cause significant damage to the boxwood at the level of the leaves, feeding exclusively on the leaf parenchyma. The presence of *Monarthropalpus buxi* larvae and the way of feeding causes the appearance of discolored galls on the surface of the leaf, mostly located near the midrib. The development dynamics of the larvae during the growing season is not uniform. This differs from one stage to another and is primarily influenced by the atmospheric temperature. studies conducted on this species showed that the egg stage of the boxwood leafminer was at an accumulation between 352-748 GDD (F), while the larval stages developed at accumulations between 679 and 2442 GDD (F) (d'Eustachio & Raupp, 2001). On the other hand, the development and survival of the

larvae is also influenced by the morphological or physiological aspects of the leaf.
Soporan et al. (2012) showed that the larvae were smaller on the leaves with less leaf surface. also, the positioning of the infested leaves in the crown of the boxwood directly influences the size of the larvae, also increasing the duration of the larval stages. Thus, in poorly lit leaves or placed in the lower part of the branches, the smallest larvae develop and have a long development period. This highlights the relationship between the physiological processes of the plant and the development of *Monarthropalpus buxi* larvae. The intense chlorophyll assimilation provides a rich source of food for the larvae and their development takes a shorter period.
Frequent and persistent attacks caused by this pest cause the death of branches and the susceptibility of plants to biotic or abiotic stressors. Cultural management is very important in the control of this pest and involves both monitoring methods by capturing adults, as well as chemical control methods by applying systemic or contact insecticides. The most recent studies have focused on chemical control, by applying insecticides, since there are few natural enemies of this pest, and biological control is significantly reduced (Raupp et al., 2004; Dunn & Saunders, 2014; Yoder et al., 2022).
The susceptibility of *Buxus* sp. before the attack of this pest causes significant economic losses in the production of boxwood. The newly formed plants are very vulnerable, regardless of the cultural practices approached in nursery technology.
The aim of this paper was to monitoring the evolution and ecology of *Monarthropalpus buxi* according to the local biotope and testing two insecticides in order to control the pest in an ornamental plant nursery from Iași County. Being a species reported for the first time in the North-Eastern area of Romania, the results obtained will constitute an additional source of documentation for further research.

MATERIALS AND METHODS

The study was carried out in two consecutive years, 2022 and 2023 in a production subunit of National Forest Administration - ROMSILVA. The location of the ornamental plant nursery is in the North-Eastern part of Romania, in the metropolitan area of the Iași city, namely Ornamental Plant Nursery – Galata, (47°14´N and 27°55´E).
The natural environment where the research took place is located in the forest steppe of Moldova on a geomorphological unit characterized by a cambic chernoziom soil. According to the Köppen-Geiger classification, the climate of this area was considered Dfb type with an average multiannual temperature of 10.2°C and a significant rainfall of 562.6 mm, distributed evenly throughout the year. The climatic factors were monitored with the meteorological FieldClimate system, located in the experimental field.
The biological material includes production fields with boxwood varieties of different ages. All samplings were multiply vegetatively, planted in the production fields at distances of 0.5 m × 0.7 m. During the growing season, maintenance of the fields was carried out with soil mobilization, fertilisation and irrigation typical for nursery production technologies.
In order to obtain conclusive results regarding the appearance and evolution of *Monarthropalpus buxi*, the observation, recording and statistical processing of data were used as research methods. During the experimental period the appearance of adults was monitored using colored glued traps: white, yellow and blue. The traps were placed every year on April 20th. from the first appearance of the adults, they were observed and inventoried daily until the end of May.



Figure 1. The placement of traps in the experimental fields

To predicting pest activity and timing control tactics were used Growing Degree-Day models. To calculated GDD for monitoring the evolution of *Monarthropalpus buxi* was used the followed mathematical equation:

$$GDD=\frac{Tmax+Tmin}{2}-threshold\ temperature,$$

where T_{max} and T_{min} are daily max and minim temperatures, threshold temperature= 10°C (Davidson and Raupp, 1999).

The GDD calculation started on March 1, because in the monthly averages of January and February they did not exceed the biological threshold of this species. According to GDD, the development of the insect's life stages was monitored. To determine the evolution of larvae stages were analysed in the laboratory thirty infested leaves, during vegetation period.

Another aspect pursued in the study was the establishment of the effectiveness of two insecticides applied during the flight period of the adults. For testing, two insecticides were compared: acetamiprid (Mospilan 20 SG) in a concentration of 0.15% and lambda-cyhalothrin (Karate Zeon) in a concentration of 0.015%. The substances were applied to the plot of boxwood mother plants, and the effectiveness was measured taking into account the dynamics of the population level before and after the treatment. They were monitored with the help of glued traps, their number being evaluated 24 hours, 48 hours and 7 days after the treatment. The obtained results were interpreted statistically.

RESULTS AND DISCUSSIONS

Boxwood (*Buxus* spp.) is part of the category of ornamental species known for its adaptability to different growing conditions, which makes it a versatile choice for landscaping. Climate, in relation with pedological factors, represent the parameters determinants in increasing or decreasing the productivity of agro-ecosystem. The temperature influences the growth and development of boxwood plants. Different species and cultivars have varying temperature requirements and tolerances (Hanaka et al., 2021; Peng et al., 2021). While some boxwoods varieties are cold-hardy and can withstand freezing temperatures, others are more sensitive to frost and require milder climates. Extreme heat can also stress boxwood plants, especially if they are not adequately watered (Ingram et al., 2015).

Adequate moisture is essential for the growth of boxwood, especially during the establishment phase. Boxwood plants prefer consistently moist but well-drained soil. In regions with low rainfall, supplemental watering may be necessary to maintain soil moisture levels, particularly during dry periods. However, excessive waterlogging can lead to root rot and other problems, so it's essential to ensure proper drainage (Helaly, 2017).

In first table (Table 1) were presented the monthly average temperature and total rainfall during the studied period. The deviation from the thermal multiannual average was also calculated. Thus, in the investigation period (2022-2023) the average annual temperature was 12.0°C with a positive deviation of 1.5°C compared to the multiannual average (10.5°C). The accumulated monthly precipitation during the studied period recorded a value of 363.6 mm, 154.0 mm less than the multiannual limit. In general, rainfall was unevenly distributed throughout the year, with a minimum in January of 9.4 mm and a maximum in July of 59.7 mm.

Table 1. Monthly and annual average of the thermal and rainfall (Ornamental Plant Nursery - Galata, Iași, 2022-2023)

Month	Air temperature (°C)			Precipitation (mm)		
	Av.	Multiannual	Deviation	Sum	Multiannual	Deviation
I	1.6	-1.9	+3.5	9.4	35.5	-19.6
II	2.7	-1.2	+3.9	16.9	32.1	-10.6
III	5.0	4.7	+0.3	31.4	71.2	+3.3
IV	9.9	11.4	-1.5	46.5	51.4	+6.2
V	16.4	17.0	-0.6	11.2	71.1	-41.3
VI	21.3	20.5	+0.8	17.3	82.9	-57.8
VII	23.3	22.4	+0.9	59.7	64.7	-9.6
VIII	24.0	21.9	+2.1	38.6	50.8	-19.0
IX	17.7	16.3	+1.4	40.3	36.5	-0.5
X	13.5	10.1	+3.4	19.3	34.4	-15.1
XI	6.7	5.4	+1.3	55.1	33.4	20.5
XII	1.7	0.1	+1.6	18.1	30.6	-10.8
Av./Sum	**12.0**	**10.5**	**+1.5**	**363.6**	**517.6**	**-154.0**

Even if in the experimental fields of the nursery this water deficit was regulated with the help of irrigation, recent studies show that deficit irrigation can be a strategy for controlling the growth of ornamental plants and increasing their ability to adapt to drought conditions (Sánchez-Blanco et al., 2019).

From another perspective, climatic factors also influence specific pests of boxwood plants. The climatic factors interact with various ecological variables to shape the evolution and population dynamics of *Monarthropalpus buxi*. Seasonal variations in temperature and day length influence the phenology of *Monarthropalpus buxi.* The insect's activity peaks during the warmer months when host plants are actively growing and producing new foliage. Winter dormancy or diapause may occur in colder climates, allowing the insect to survive adverse conditions until favorable conditions return (Raupp et al., 2002).

Figure 2 highlight the flight season adults of *Monarthropalpus buxi* in 2022 and 2023, monitored and captured using colored traps. Under the conditions of 2022, the first adults were captured on 9th May on the white panels, and in the following days, colored traps were also occupied. The maximum flight curve was on 17th May when 1108 adults were captured (723 adults on white traps; 288 adults on yellow traps and 97 adults on blue traps). After that date, the number of adults gradually decreased, and the last ones captured were on 28th May. In 2023, spring temperatures were higher, causing the pest to appear earlier. The first adults were captured on 2nd May on the white panels, on 4th May on the yellow panels and on 5th May on the blue panels. The maximum flight curve was on 13th May, when the largest number of captures was obtained – 1471 adults. Their last flight was recorded on 22th May 22 on the blue panels, and adults were captured on the other two until 27th May. During the study period, a total of 12999 adults were captured.

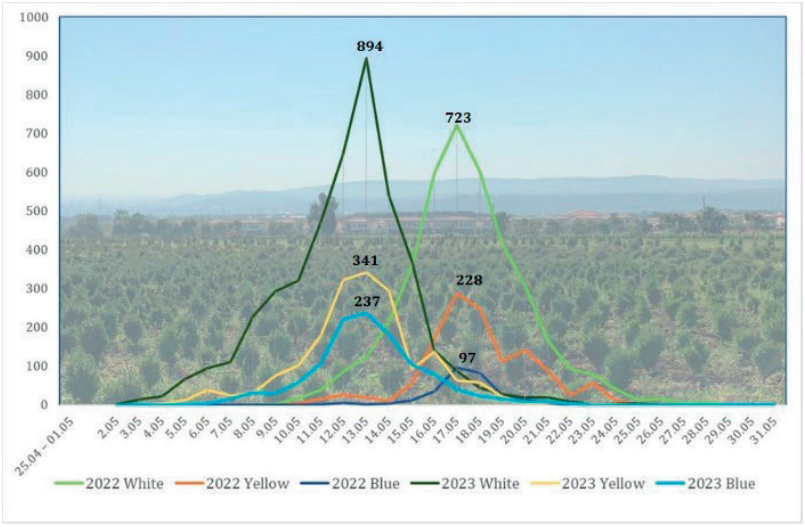


Figure 2. The flight curve of adults captured during the study period

The study on the capture of adult populations using chromotropic traps of different colors, carried out within the ornamental plant nursery, during the period 2022-2023, highlights significant variations in their effectiveness, influenced by the behavioral preferences of the studied species and by local abiotic factors (Table 2). Comparative analysis of empirical data demonstrates that, in both experimental seasons, white traps had a significantly higher attractiveness than yellow and blue ones, capturing a much higher number of individuals, with an increase from 185.9% compared to the control in 2022 to 204.5% in 2023. In contrast, yellow traps showed an intermediate effectiveness, oscillating between 58.7% of the control value in 2022 and 83.7% in 2023, suggesting a variable sensitivity of

Monarthropalpus buxi adults to this chromatic spectrum, correlated with seasonality and abundance of available trophic resources.

On the other hand, blue traps proved considerably less effective, recording only 13.2% of the control value in 2022 and a modest increase to 54% in 2023, which indicates a low receptivity of insects to this chromatic spectrum, according to previous studies on the negative phototactism of some entomological species to blue light (Otieno et al., 2018). The observed differences are supported by statistical analyses, with white traps recording highly significant differences ($p < 0.01$ in 2022 and $p < 0.001$ in 2023), while the efficiency of yellow and blue traps did not reach relevant statistical significance thresholds, being labeled as "Mt" (non-significant).

Also, comparing the results obtained, it is observed that following the application of the Duncan Test there are significant differences between the variants.

Table 2. The level of adult populations captured with colored traps (n=6, Ornamental Plant Nursery - Galata, Iași, 2022-2023)

Colour traps	Year	Samples	% of control	Difference to control	The semnificance of difference	Duncan Test [1]
White	2022	4027	185.9	+1861	**	A
Yellow		1272	58.7	-895	Mt	C
Blue		287	13.2	-1880	oo	D
White	2023	4430	204.5	+2264	***	A
Yellow		1814	83.7	+645	Mt	b
Blue		1169	54.0	-998	Mt	c
Average (control)		2167	100	-	-	-
DL (P 5 %) – 1013.82; DL (P 1 %) – 1423.07; DL (P 0.1 %) – 2009.04						

[1]different letters correspond with the significant statistical difference for $P \leq 5\%$, Duncan test.

The development of boxwood leafminer (*Monarthropalpus buxi*) in relation with GDD accumulations was reported in Table 3. The boxwood leafminer adults developed at accumulations between 196.2-329.7 GDD in 2022, and in 2023 they appeared following accumulations of 191.7-324.6 GDD. The first eggs were observed at 329.7 GDD in 2022 and next year at 324.6 GDD. This identification was observed on the new growth leaves. The larval stages evolved between 587.9-1298.3 GDD in 2022. The following year accumulation degree were included between 324.6-1317.1 GDD. The transformation of 4th larvae stage into pupae requires a low temperature stage, below 0°C. Therefore, for this period was accumulated minimum 98.6-196.2 GDD (2022) and 117.9- 191.7 (2023).

Table 3. The evolution of *Monarthropalpus buxi* in relation to the accumulation of Growing Degree-Days - GDD (°C)

No.	Life stage	Year	GDD (°C)			Period
			First occurance	Peak occurance	Last occurance	
1.	Pupa	2022	98.6-196.2			April -May
		2023	117.9-191.7			
2.	Adult	2022	196.2	262.7	329.7	May
		2023	191.7	246.3	324.6	
3.	Egg	2022	329.7-587.9			May -June
		2023	324.6-602.1			
4.	1st instar	2022	587.9-675.7			June
		2023	602.1-670.4			
5.	2nd instar	2022	675.7-984.3			July- September
		2023	670.4-991.5			
6.	3rd instar	2022	984.3-1298.3			September- March
		2023	991.5-1317.1			
7.	4th instar	2022	0-98.6			March -April
		2023	0-117.9			

Figure 3 shows the life stages of the boxwood leafminer determined in the laboratory during the study period. Another aspect pursued in the study was testing the effectiveness of two insecticides to combat the pest. Table 4 shows the results obtained in the two consecutive years regarding the distribution of *Monarthropalpus buxi* adults before and after the application of the treatment. In combating boxwood leafminer (*Monarthropalpus* spp.), both systemic and contact insecticides can be effective depending on the severity of the infestation and the specific circumstances of the situation.



Figure 3. Life stages of *Monarthropalpus buxi* determined in the study period

In our study we obtained a high efficiency of the two substances: acetamiprid registering in the two years an efficiency between 95.6-100%, and the efficiency of lambda-cyhalothrin registered values between 97.8-99.7%

Table 4. The effectiveness of insecticide application in combating *Monarthropalpus buxi*

		V1				V2			
		Acetamiprid				*Lambda-cyhalothrin*			
		2022	Eff.(%)	2023	Eff.(%)	2022	Eff.(%)	2023	Eff.(%)
No of captures before treatment		478	-	623	-	365	-	557	-
No of captures after treatment	24 hours	21	95.6	18	97.1	7	98.1	12	97.8
	48 hours	13	97.2	9	98.5	3	99.1	5	99.1
	7 days	2	99.9	0	100	1	99.7	2	99.6

CONCLUSIONS

Boxwood leaminer (*Monarthropalpus buxi*, Diptera, Cecidomyidae) has become a key pest of boxwood varieties in recent years, causing damage to the general appearance of the plant and also affecting the photosynthetic activity of the leaves.

The climatic factors interact with various ecological variables in the evolution and population dynamics of *Monarthropalpus buxi*. Seasonal variations in temperature and day length influence the phenology of *Monarthropalpus buxi*. In the conditions of North-Eastern part of Romania, the life cycle of *Monarthropalpus buxi* was monitored in relation to GDD. The first adults appeared between 191.7-196.2 GDD (°C), and the maximum flight time was at accumulations between 246.3-262.7 GDD (°C). Larval stages developed between 670.4-1317.1 GDD (°C).

Combating this pest is effective by combining physical methods (colored calpans) with chemical methods (insecticides). During the study period, a total of 12999 adults were captured, the most catches being registered on the white traps. In combating boxwood leafminer (*Monarthropalpus* spp.), both systemic and contact insecticides can be effective depending on the severity of the infestation and the specific circumstances of the situation.

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IN VITRO ANTIFUNGAL EFFECT OF *ROSMARINUS OFFICINALIS* L. ESSENTIAL OILS AGAINST *FUSARIUM* SP.

Ionuț-Daniel MARIN[1], Elena Ștefania IVAN[2], Monica-Luminița BADEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: elena.ivan@qlab.usamv.ro

Abstract

The in vitro antifungal activity of essential oils extracted from Rosmarinus officinalis L. was evaluated against Fusarium graminearum, a pathogen isolated from infected maize (Zea mays) plants. Three essential oil samples were tested: one obtained by laboratory distillation and two commercial products. Antifungal activity was assessed using two methods: incorporation of the oils into the culture medium to quantify mycelial growth inhibition, and application of filter paper discs on PDA medium to observe localized fungal inhibition. All three essential oils exhibited inhibitory effects on the growth of Fusarium graminearum, with the laboratory-extracted oil showing the highest efficacy. The incorporation method provided quantitative data on mycelial growth reduction, while the disc method served as a qualitative complementary approach. These results highlight the potential of R. officinalis essential oil, particularly when freshly extracted, as a natural antifungal agent against Fusarium graminearum affecting maize crops.

Key words*: Rosmarinus officinalis; Fusarium spp; maize; antifungal activity; in vitro.*

INTRODUCTION

Essential oils (EO) are volatile extracts primarily obtained from aromatic and medicinal plants. They serve as a rich source of biologically active compounds with antibacterial, insecticidal, fungicidal, nematicidal, herbicidal, antioxidant, and anti-inflammatory properties (Bakkali et al., 2008; Valerio et al., 2021). Historically, medicinal and aromatic plants (MAP) have been used to produce essential oils, mainly for flavoring and fragrance purposes, rooted in the tradition of using aromatic plants as culinary herbs and spices (Buckle, 2014). Among the plants with culinary and medicinal uses (Vâșcă-Zamfir et al., 2018), we mention the species *Rosmarinus officinalis* L. (Lamiaceae), also known for its antibacterial and antifungal properties. Over time, their applications have expanded into medicine, where they are now widely used in phytopharmaceuticals and aromatherapy (El Asbahani et al., 2015).

Beyond their physiological roles, these volatile compounds also perform essential ecological functions. They can act as internal signaling molecules, natural defense mechanisms against herbivores, or attractants for pollinators (Hedden et al., 2002). More recently, research has shifted toward their potential as biocontrol agents, employing low concentrations of EO to manage plant pests and pathogens (Isman, 2020; Andrés et al., 2012). This has positioned them as a promising alternative to synthetic pesticides, offering sustainable solutions for crop protection and pest control (Prabha et al., 2016). The increasing interest in EO applications has driven extensive academic and industrial research into their biological activities (Turek & Stintzing, 2012; Bassolé & Juliani, 2012). Biocontrol, a growing concept in pest management, has gained attention as part of integrated pest management (IPM) strategies (Barzman et al., 2015). The shift toward eco-friendly biopesticides is seen as a viable alternative to synthetic chemicals, reducing environmental contamination while maintaining agricultural productivity (Isman, 2019).

Numerous studies have demonstrated the biopesticidal potential of essential oils, particularly their antifungal, insecticidal, and nematicidal effects (Raveau et al., 2020; Arraiza et al., 2018). However, their direct use as

biopesticides presents several challenges, including phytotoxicity-long considered a major limitation in their development as insecticides or fungicides (Werrie et al., 2020) - their impact on food organoleptic properties, and the need for precise application rates to ensure efficacy (Turek & Stintzing, 2013).

Furthermore, their high volatility and susceptibility to degradation reduce their effectiveness when applied directly in the field (Turek & Stintzing, 2013). Although some commercial products based on essential oils-such as orange oil or essential oil blends-are already available on the market, further research is still needed to optimize their use in integrated biocontrol strategies and to expand their application to a wider range of pathogens and crops (Ravensberg, 2015).

The chemical composition of essential oils varies significantly across regions and even within the same geographical area due to differences in environmental conditions (El Asbahani et al., 2015; Turek & Stintzing, 2012), as well as depending on the phenophase and the parts of the plant (Badea et al., 2021). Addressing these variations and improving EO formulations will be crucial for their broader adoption in sustainable agriculture.

The phenological stage of the plant significantly influences EO yield, which differs among species (Tamokou et al., 2017). Typically, EOs from medicinal and aromatic plants are extracted during the flowering stage, prior to seed germination. However, this practice can hinder plant regeneration, making high-yield extraction challenging and underscoring the need for the domestication of medicinal and aromatic plants (MAPs) (Isman, 2019).

Unregulated harvesting of MAPs often leads to habitat loss, putting pressure on wild plant populations (Barzman et al., 2015). This issue is particularly critical in developing countries such as India, China, Nepal, Kenya, Tanzania, and Uganda, where the decline of medicinal plant species has accelerated (Bakkali et al., 2008). To meet the rising demand for standardized, high-quality raw materials in industrial markets, medicinal and aromatic plants have been systematically domesticated and cultivated (Isman, 2019). Additionally, many wild plants with human uses are now being cultivated as Crop Wild Relatives (CWR) to support conservation efforts (Valerio et al., 2021).

The aim of this study was to evaluate the in vitro antifungal activity of essential oils extracted from *Rosmarinus officinalis* L. against *Fusarium* spp., by comparing the efficacy of a laboratory-extracted oil with two commercial samples using two testing methods: incorporation into culture medium, which provided quantitative data on mycelial growth inhibition, and the filter paper disc method, which offered qualitative observations of fungal growth suppression.

MATERIALS AND METHODS

The biological material studied consisted of *Rosmarinus officinalis* L. plants grown in the medicinal and aromatic plant garden of the Faculty of Agriculture, specializing in Biology.

The essential oil from the rosemary samples was extracted using a Clevenger-type apparatus. The pre-prepared rosemary samples were distilled in water for three hours before the boiling process began. Approximately 400 g of fresh rosemary plant material (in the flowering phenophase) was used for the oil extraction, maintaining a 1:3 plant material-to-water ratio.

The antifungal activity of the essential oils from *Rosmarinus officinalis* L. was tested at the Research Center for the Study of Agro-Food Product Quality, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The antifungal effects were compared to other commercially available essential oils, with the following experimental variants:

Pure, natural rosemary essential oil;

Commercial rosemary essential oil labeled as "S1";

Commercial rosemary essential oil labeled as "S2".

The microbiological material used for the experiment was *Fusarium* sp., isolated from *Zea mays* L., *Fusarium* spp. represents a complex of phytopathogens that colonize plant organs, particularly attacking seeds, causing rot, tracheomycosis, qualitative deterioration, yield reduction, and, in the case of *Fusarium graminearum*, mycotoxin contamination that affects the food chain (Cristea et al., 2024).

To evaluate the *in vitro* antifungal efficacy against Fusarium sp. isolated from corn cobs, two methods were used: the incorporation into culture medium (Food Poison Technique) to assess mycelial growth inhibition quantitatively, and a modified Kirby-Bauer disc diffusion method, using filter paper discs impregnated with essential oil at the tested concentrations. The inclusion method was applied at a concentration of 1%, chosen as a preliminary dose based on previous studies reporting antifungal effects of *Rosmarinus officinalis* essential oil. The essential oils were emulsified in 0.1% Tween 80 to enhance dispersion in the culture medium. The antifungal effectiveness was evaluated by measuring the radial growth of *Fusarium* sp. mycelium on treated plates compared to untreated controls. The percentage of mycelial growth inhibition was calculated using the formula: Inhibition (%) = C-T/C X 100.

Where C is the average colony diameter in the control, and T is the average diameter in the treated variant

The diffusion method with filter paper discs was applied, using a protocol adapted from the Kirby-Bauer method. Discs were impregnated with the same 1% essential oil solution used in the inclusion method, then placed on solidified PDA medium previously cooled to 45°C.

Fungal discs (7 mm diameter) were collected from the edge of actively growing Fusarium sp. cultures using a sterile cork borer. Petri dishes containing PDA medium were first inoculated centrally with a fungal disc, after which filter paper discs impregnated with 10 µL of rosemary essential oil (1% concentration) were placed at a distance of approximately 40 mm from the fungal inoculum. This distance was selected to allow clear observation of potential inhibition zones, based on modifications of standard disc diffusion protocols (Bauer et al., 1966; adapted for filamentous fungi).

In the experiments assessing the antifungal activity of rosemary essential oils, the fungal strain (Fusarium sp.) was incubated at 25 ± 1°C in darkness for 3, 6, and 9 days on PDA medium supplemented with a 1% essential oil solution emulsified in 0.1% Tween 80. The same amount of emulsifier was added to the control plates without essential oil. Fungal growth was monitored by measuring colony diameter with a graduated ruler, allowing for the assessment of the strain's sensitivity to the tested oils.

Petri dishes containing PDA medium without essential oil were used as control variants to evaluate the normal growth of the fungal strain in the absence of treatment (Figure 1).

The antifungal activity of rosemary essential oil was assessed by measuring the radial growth of *Fusarium* sp. colonies at 3, 6, and 9 days. The results, presented in Table 1, show a progressive increase in mycelial growth over time. Compared to the control, the treatment with rosemary essential oil resulted in an average inhibition of 7% after 9 day.

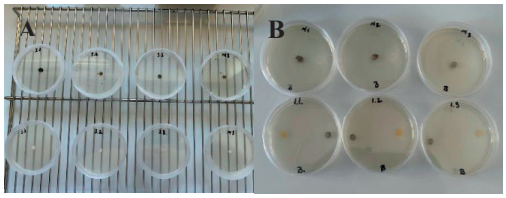


Figure 1. Method of Inclusion in Medium (A) and Diffusion Method with Filter Paper Discs (B) (original)

Table 1. Mycelial growth of *Fusarium* sp. on PDA medium (control), measured over time

Day	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean Diameter (mm)	Growth Rate (mm/day)*
3	21.5	22.5	19.0	21.0	7.0
6	46.5	45.5	46.0	46.0	7.7
9	63.5	67.0	59.0	63.2	7.0

*Growth rate calculated as mean diameter / number of days.

RESULTS AND DISCUSSIONS

In the experiments testing the antifungal activity of rosemary essential oils, the studied strain was incubated under controlled conditions for 3, 6, and 9 days on PDA culture medium supplemented with a 1% volatile oil solution. The development of the strain was monitored,

and the diameter was measured using a graduated ruler, allowing for the evaluation of the pathogenic microorganisms' sensitivity level.
Petri dishes containing PDA medium without essential oil were prepared to observe the natural development of the pathogen (Figure 2).
The treatment with pure rosemary essential oil significantly inhibited the mycelial growth of *Fusarium* sp. at all observation intervals. Compared to the control, the inhibition rates were 61.9% after 3 days, 75.7% after 6 days, and 68.7% after 9 days (Table 2 and Table 3). These results confirm the strong antifungal potential of the tested oil.

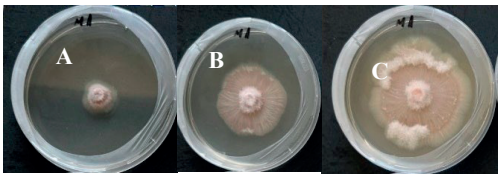


Figure 2. Evolution of the pathogen at 3 (A), 6 (B), and 9 (C) days on the control plates (sursă: original)

Table 2. Mycelial growth of *Fusarium* sp. on PDA medium supplemented with pure rosemary essential oil

Day	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean Diameter (mm)
3	12.0	12.0	0.0	8.0
6	13.0	10.5	10.0	11.2
9	29.0	13.5	17.0	19.8

Table 3. Results

Day	Mean Control (mm)	Mean EO Treatment (mm)	Inhibition (%)
3	21.0	8.0	61.9
6	46.0	11.2	75.7
9	63.2	19.8	68.7

The commercial essential oil sample S1 also showed antifungal activity, particularly at the early stage of fungal development. At 3 days post-inoculation, the inhibition reached 78.6%, followed by lower values at 6 days (42.8%) and 9 days (27.5%), suggesting a reduced long-term effect compared to the pure oil treatment (Table 4 and Table 5).

Table 4. Mycelial growth of *Fusarium* sp. on PDA medium supplemented with commercial rosemary essential oil (S1)

Day	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean Diameter (mm)
3	13.5	0.0	0.0	4.5
6	36.0	26.0	17.0	26.3
9	61.0	45.0	31.5	45.8

Tabel 5. Inhibition percentage vs. Control

Day	Mean Control (mm)	Mean S1 (mm)	Inhibition (%)
3	21.0	4.5	78.6
6	46.0	26.3	42.8
9	63.2	45.8	27.5

In repetitions 2 and 3, growth was slower, as the essential oil had inhibited pathogen development at day 3 (Figure 3).

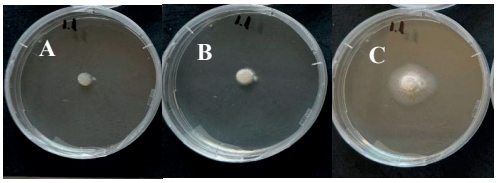


Figure 3. Evolution of the pathogen at 3 (A), 6 (B), and 9 (C) days on the plates enriched with pure essential oil (original)

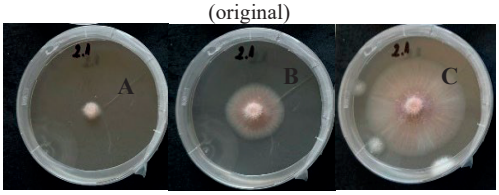


Figure 4. Pathogen development at 3 (A), 6 (B), and 9 (C) days on plates inoculated with the commercial essential oil "S1" (original)

The growth diameter recorded at 3 days (72 hours) post-inoculation with *Fusarium* spp. in the medium enriched with the commercial essential oil extract "S2" showed limited development, exhibiting a significant inhibitory effect on the plates in repetitions 2 and 3.

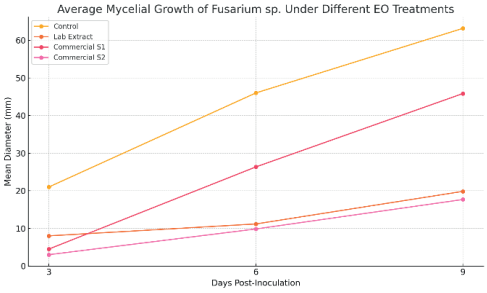


Figure 5. Comparative mycelial growth of Fusarium sp. on PDA medium supplemented with rosemary essential oils (lab-extracted and commercial samples) over 3, 6, and 9 days

Observations conducted at 6 days post-inoculation revealed growth diameters of 10 mm (replicate 1), 12 mm (replicate 2), and 7.5 mm (replicate 3), with a calculated mean of 9.83 mm. At 9 days, the recorded diameters were 15 mm, 19.5 mm, and 18.5 mm, respectively, resulting in a mean value of 17.67 mm. These data are summarized in Figure 5

Filter paper disc diffusion method

In the experiments assessing the antifungal activity of rosemary essential oils, the studied strains were incubated under controlled conditions for 3, 6, and 9 days on PDA culture medium. Petri dishes containing PDA were first inoculated with a spore suspension of *Fusarium* sp., after which sterile paper discs impregnated with 10 µL of a 1% essential oil solution (diluted in 0.5% Tween 80) were placed on the surface of the medium. The distance between the fungal inoculation point and the essential oil - impregnated disc was approximately 40 mm. The method was adapted to assess the radial diffusion of the essential oil and its potential inhibitory effect on mycelial growth.. Their development was monitored, and the growth diameter was measured using a graduated ruler, allowing for the evaluation of the pathogenic microorganisms' sensitivity. Petri dishes containing PDA medium without essential oil discs were used as control to assess the normal growth rate of the pathogen.

The fungal growth diameter was measured at 3, 6, and 9 days post-inoculation, following the same methodology applied to the plates treated with essential oil discs. Three biological replicates were performed for each experimental time point. At 3 days, the average diameters were 12.5 mm for replicate 1, 13 mm for replicate 2, and 13.5 mm for replicate 3.

On day 6, the average diameters recorded were 36.5 mm for both replicate 1 and 2, and 43 mm for replicate 3. At 9 days, the growth diameters were 53.5 mm for replicate 1, 57.5 mm for replicate 2, and 63 mm for replicate 3 (Figure 6).

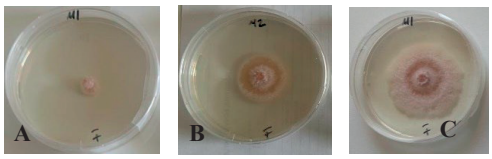


Figure 6. Pathogen evolution at 3 (A), 6 (B) and 9 (C) on control plates (original)

Seventy-two hours after inoculation with *Fusarium* spp., the growth diameters recorded on PDA medium containing discs impregnated with pure essential oil showed limited fungal development. The diameters recorded were 13.5 mm (replicate 1), 15 mm (replicate 2), and 14.5 mm (replicate 3).

At 6 days post-inoculation, the growth continued moderately, with diameters of 27.5 mm, 33.5 mm, and 35 mm for replicates 1, 2, and 3, respectively. After 9 days, further development was observed, with diameters reaching 41 mm (replicate 1), 48.5 mm (replicate 2), and 54.5 mm (replicate 3) (Figure 7).

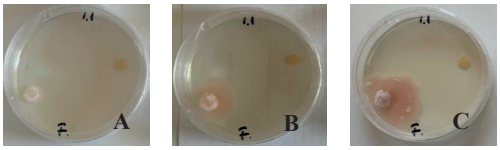


Figure 7. Pathogen evolution at 3 (A), 6 (B) and 9 (C) on discs with pure essential oil

In the experimental variant containing discs impregnated with the commercial volatile oil "S1," fungal growth was observed after 3 days of incubation. The diameters recorded were 10.5 mm (replicate 1), 14.5 mm (replicate 2), and 11.5 mm (replicate 3).

At 6 days post-inoculation, the fungal colony showed further expansion, with diameters of 37.5 mm, 46 mm, and 38 mm for replicates 1, 2, and 3, respectively. By day 9, growth continued, reaching 51 mm (replicate 1), 60 mm (replicate 2), and 57.5 mm (replicate 3) (Figure 8).

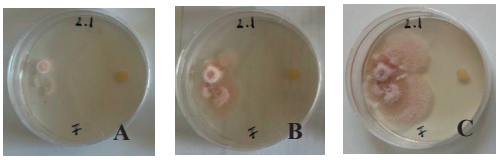


Figure 8. Pathogen evolution at 3 (A), 6 (B) and 9 (C) on discs with commercially available essential oil “S1”

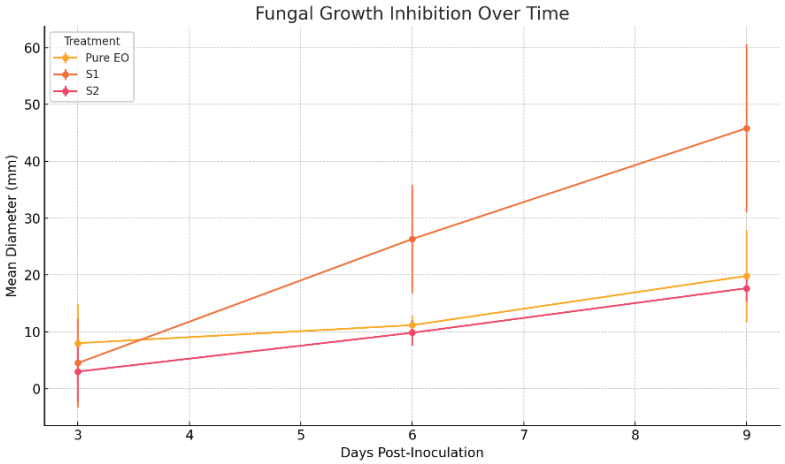


Figure 9. Comparison of *Fusarium* spp. growth on PDA medium treated with different rosemary essential oil samples over 9 days

The graph clearly illustrates that the pure essential oil had the strongest antifungal effect, with significantly lower growth diameters across all time points, while the commercial sample S1 showed a progressive increase in fungal growth, suggesting lower efficacy compared to the pure and S2 samples (Figure 9).

The growth diameter recorded at 72 hours post-inoculation with *Fusarium* spp. in the medium containing discs impregnated with the essential oil extract "S2" ranged from 11 mm (replicate 1) to 13.5 mm (replicate 3), with a mean of 12.3 mm. After 6 days, the mean growth diameter was 30.3 mm, with individual values of 30 mm, 28.5 mm, and 32.5 mm for replicates 1, 2, and 3, respectively. At 9 days post-inoculation, the recorded growth diameters were 43 mm, 36.5 mm, and 47 mm, with a calculated mean of 42.2 mm (Figure 10).

The comparative analysis of the incorporation method using pure rosemary essential oil and two commercial formulations, referred to as "S1" and "S2", revealed significant differences in antifungal activity, likely linked to oil

composition and quality. The term pure essential oil refers to the laboratory-distilled Rosmarinus officinalis oil with no additives or diluents, while "S1" and "S2" are commercial products with unspecified concentrations and possible carriers or solvents.

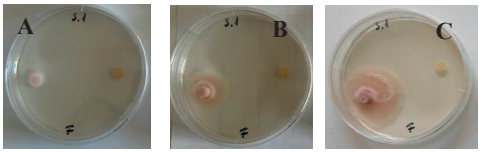


Figure 10. Pathogen evolution at 3 (A), 6 (B) and 9 (C) on discs with commercially available essential oil “S2”

At 3 days post-inoculation, the average growth diameter on PDA supplemented with pure EO was 8 mm, compared to 4.5 mm for S1 and 3 mm for S2. By day 6, the pathogen growth increased to an average of 11.1 mm on pure EO plates, while for S1 it reached 26.3 mm and for S2, 9.8 mm. These results are in agreement with previous studies (Babar et al., 2018; Hussain et al., 2016), which reported higher antifungal effectiveness of undiluted rosemary EO due to its high content of 1,8-cineole, camphor, and α-pinene. Differences between samples may be attributed to variations in oil purity, plant origin, or formulation.Pure essential oil demonstrated its capacity to inhibit pathogen growth throughout the entire 9-day period, with an average diameter of 19.8 mm across the 3 repetitions. In comparison, the "S1" sample recorded an average diameter of 45.8 mm, and the "S2" sample reached an average of 53 mm. The control group showed an average diameter of 63 mm across the 3 repetitions.

These results suggest a notable effectiveness of the 1% solution prepared from pure rosemary essential oil in suppressing the pathogen throughout the observation period. These results suggest that, despite a slower start, pure essential oil exerts a remarkable antifungal effect, making it a viable alternative for managing infections caused by *Fusarium* spp.

The comparative analysis of the diffusion method using filter paper discs between the solution prepared from pure rosemary essential oil (1%) and two commercial oil samples, "S1" and "S2", revealed significant differences in antifungal efficacy. After 3 days, the commercial oils "S1" and "S2" showed slightly higher inhibition, with average growth diameters of 12.16 mm and 12.33 mm, respectively, while the pure essential oil recorded 13.3 mm and the control 13 mm. At day 6, the lowest diameter was observed for "S2" (30.33 mm), followed by the pure essential oil (32 mm), while "S1" and the control presented higher values (36.83 mm and 38.66 mm, respectively). By day 9, although the inhibitory effects decreased overall, "S2" maintained the lowest growth diameter (42.16 mm), followed by the pure oil solution (48 mm). "S1" and the control had the largest diameters (54.16 mm and 58 mm). These results suggest that "S2" and the pure essential oil solution exhibited more sustained inhibitory effects against *Fusarium spp.* compared to "S1" and the control (Figure 11).

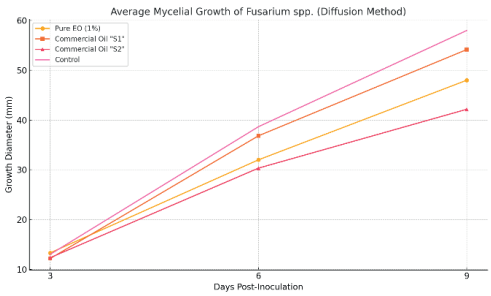


Figure11. Average growth diameter (mm) of Fusarium spp. over 9 days post-inoculation under different treatments using the diffusion method. Commercial oil 'S2' and pure essential oil (1%) demonstrated greater inhibition compared to 'S1' and the control

CONCLUSIONS

In conclusion, the study demonstrated that both the pure rosemary essential oil and the commercial oil "S2" exhibited superior and sustained inhibitory effects on *Fusarium spp.* when compared to the "S1" oil and the untreated control. Although the pure oil showed a slower inhibitory onset, it maintained notable antifungal activity throughout the observation period, indicating potential as an alternative agent in pathogen management.

These results may be attributed to differences in chemical composition among the oils, as the antifungal effectiveness of essential oils is closely linked to their major constituents (e.g., 1,8-cineole, camphor, α-pinene) and

chemotypes. While gas chromatography–mass spectrometry (GC-MS) analysis was not conducted in this study, such characterization is essential in future work to correlate bioactivity with specific chemical profiles. The findings suggest a need for further research to identify the active compounds responsible for the observed antifungal effects and to establish standardized formulations for practical applications.

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DIFFERENCES IN MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE AMONG TWO VERMICOMPOST GRANULATIONS

Gabi Mirela MATEI[1], Sorin MATEI[1], Laurențiu Liviu URECHESCU[2], Elena Maria DRĂGHICI[2]

[1]National Research and Development Institute for Soil Science, Agrochemistry and Environment, Bucharest, 61 Mărăşti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: so_matei602003@yahoo.com

Abstract

This paper presents the results of the research concerning the influence of two vermicompost granulations, C1<2 mm and C2>2 mm, obtained by sieving, on bacterial and fungal diversity and community structure. Both bacteria and fungi have colonized the vermicompost granulation C2>2 mm with higher counts as compared with C1 fraction. Global microbial activity was expressed by higher values of soil respiration in C2 granulation (123.809 mg CO_2 x 100 g^{-1}soil) as compared to 116.833 mg CO_2 x 100 g^{-1}soil in C1 granulation. A rich bacteria population with high diversity (H'=2.104 bits) and homogeneity (ε=0.662) was found in C2 granulation. C1 granulation contained a less diverse bacteriobiome, with H'=1.750 bits and ε=0.594. Fungi presented lower biodiversity values than bacteria. Conditions from C1 were more favourable than those from C2 granulation (H'=0,921 bits, ε=0.324 in C1 and H'=0.403 bits, ε=0.089 in C2 fraction). Bacterial microflora from C1 granulation was dominated by Bacillaceae, accompanied by Pseudomonas and actinomycetes and a mix of species (Bacillus, Micrococcus, Pseudomonas, Arthrobacter and actinomycetes) in C2 granulation. In both fractions identified strong cellulolytic species (Fusarium, Scopulariopsis, Penicillium, Cladosporium).

Key words*: vermicompost; granulation; bacteria; fungi; microbial communities; biodiversity.*

INTRODUCTION

Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa (Banerjee et al., 2019). The restoration of microbial diversity in agricultural soils needs utilization of various natural amendments and fertilizers for improving soil physical, chemical and biological properties.
On the other hand, research look for sustainable alternatives for management of enormous quantities of organic wastes, with potential benefits for both the environment and agricultural economics. One of them refers to decomposition of organic wastes by vermicomposting, the final product resulting from this process is the vermicompost, an organic biofertilizer utilisable for its beneficial effects on soil properties and plant growth and health. Vermicomposting is defined as the process of biodegradation and stabilization of organic materials, facilitated by the collaborative efforts of earthworms and mesophilic microorganisms. While microorganisms play a vital role in the biochemical breakdown of organic matter, earthworms contribute by fragmenting and conditioning the substrate (conferring a good aeration and increasing the surface area), which induces significant changes in its microbiota and its biological activity, thus accelerating substrate decomposition (Madhushani et al., 2024). Epigeic earthworms are generally used for organic waste decomposition and they consume microorganisms specially fungi to satisfy their nitrogen requirement, thus modulating microbial communities and biosynthesis of Plant Growth Promoting Biostimulants (PGPBs). In addition to high macro-nutrient content, the microbial properties of vermicompost providing plant growth-stimulating capability could be attributed to the PGPBs, which include humification substances (HS), particularly fulvic acid, components with plant hormones activity (auxin, cytokinin, gibberellin, etc.), protein hydrolysates,

betaines. Increase in microbial biomass and diversity of beneficial microflora (e.g. plant growth-promoting bacteria, actionmycetes and fungi) indicated that vermicomposting facilitates microbial proliferation in final stabilized product (Esakkiammal et al., 2015).

Another recent study on the vermicompost microbiota and the effect of PGPBs on plants growth and health confirmed the biosynthesis of phytohormones: indolil acetic acid (IAA), jasmonic acid (JA) and salicylic acid (SA), and the production of HS (especially fulvic acid), responsible for auxin-like activity of vermicompost (Zhang et al., 2024).

It is well-known that earthworm castings are enriched in main nutrients and calcium humate, the binding agent that prevents desiccation and contributes to the incubation and proliferation of beneficial microbial species, such as *Trichoderma* spp., *Pseudomonas* spp. and vesicular-arbuscular mycorrhizal species. A wide variety of wastes can be utilized as substrates for degradation with various earthworms and microorganisms. Manyuchi et al. (2012) vermicomposted the waste corn pulp blended with cow dung using *Eisenia fetida*. Rupani et al. (2023) vermicomposted green organic wastes using *Eisenia fetida* under field conditions.

Achsah & Prabha (2013) utilized vermicompost produced from banana waste to improve the growth parameters of tomato plants. Vermicomposting of paper cup wastes is a source of cellulolytic bacteria with high ability to produce lytic enzymes for accelerated cellulose decomposition (Karthika et al., 2020). Research on vermicompost microflora and beneficial effect for plants carried out by Maji et al. (2017) found that humic acid rich vermicompost promotes plant growth by improving soil structure, aeration conditions, diversity and biomass of microbial community, root nodulation and mycorrhizal colonization in the roots of peas. Yao et al. (2023) presented the complex role and mechanisms of action of beneficial fungi from genus *Trichoderma*, frequently identified in vermicomposts. Its biological control mechanism of plant fungal and nematode disease included competition, antibiosis, antagonism and mycoparasitism (Kumar et al., 2016), as well as the mechanism of promoting plant growth, inducing plant systemic resistance and inactivation of the pathogen's enzymes involved in the infection process (Singh et al., 2018; Yan et al., 2021). *Trichoderma* species were reported to produce volatile organic compounds responsible for antimicrobial activity, antibiotics, various enzymes (Joo & Hussein, 2022). Detritivorous earthworm activity stimulates microbial communities to use more efficiently the available energy, shaping their structure in organic wastes during vermicomposting. Earthworms are able to accelerate the decomposition of organic matter by modulating the decomposer community composition in the short term. Different vermicomposts can be produced by using different earthworm species and different types of organic wastes containing huge and specific microbial composition. Thus, it is possible to obtain specific vermicomposts utilisable for different practical applications.

In a recent study, Manzoor et al. (2024) strongly recommended the vermicompost as potential organic fertilizer for sustainable vegetable cultivation for the multiple benefits, such as: a slow-releasing organic manure rich in humic substances (fulvic acid, humic acid, and humin); enhancing microbial diversity, enzymatic activity; providing a sustained supply of macro and micronutrients; improving their retention and absorption due to its high porosity, water-holding capacity, and aeration; stimulating plant growth and suppressing the phytopathogens. Similar results reported by Iqbal et al. (2024) presented the beneficial effect of vermicompost application in alleviating the Cd toxicity by enriching the soil with beneficial microbes, enzymes, and humic acids, improving soil structure and water retention capacity, enhancing soil health, plant physiological and antioxidant defence mechanisms, in fragrant rice. Vermicomposting may contribute to soil organic carbon (SOC) sequestration in aggregates by bacterial necromass-C and with 43.96% higher values from fungal necromass-C (Zang et al., 2023). Barik et al. (2010) reviewed the results concerning the production of vermicompost from various agricultural wastes, the soils treated with vermicompost presenting higher microbial loads (e. g. nitrogen fixing bacteria in paddy fields) than in untreated plots.

By improving soil physical, chemical and microbial properties, increasing the number and biodiversity of microorganisms, vermicomposts can be utilized in sustainable agriculture and in soil restoration (Trivedi et al., 2021). A recent study in China on the effects of vermicompost application on the soil microbial community structure and fruit quality in melon (*Cucumis melo*) reported enhancement of soil chemical properties, increasing enzyme activity, and diversity of microbial communities, as well as chlorophyll content and melon quality (Tian et al., 2024). Lim et al. (2015) recommended the use of vermicompost as an effective organic fertilizer and biocontrol agent in organic farming for its beneficial effects on soil, yields and economy. The process of vermicomposting is self-sustaining, self-regulating, and self-improving technology. It requires minimal or no energy input, being considered a low-cost process and an environmentally friendly waste management option (Abad & Shafiqi, 2024). Vermicompost obtained is an easily available organic fertilizer comparatively with inorganic fertilizers, and a sustainable green solution for organic waste management problems (Arancon et al., 2006; Madhushani et al., 2024).

Few authors (Pocius et al., 2014) investigated the possibility to obtain granulated vermicompost and assessed the influence of granulation process parameters on granulated fertilizer properties.

Research has been carried out aiming to compare the bacterial and fungal diversity, community structure, microbial activity and quality of two vermicompost granulations, C1<2 mm and C2>2 mm, obtained by sieving.

MATERIALS AND METHODS

The experiment was carried out in laboratory conditions at the Faculty of Horticulture from University of Agronomic Sciences and Veterinary Medicine of Bucharest. The biological material represented by food scraps, compost and different organic inputs (coco coir, hay, dead leaves, crushed egg shells, coffee grounds) and 100 red worms from species *Eisenia andrei* (California Red Wigglers) per tray was composted at the room temperature of 20^{o}C over a period of 47 weeks. The study involved testing each variant with three replicates, to produce vermicompost in trays.

The vermicompost obtained had a neutral pH (7.19) and high humus content (50.12%). A complete description of the experiment and the results of chemical parameters of the vermicompost were presented elsewhere (Urechescu et al., 2024). The values exceeded the target with more than 15 times for humus, 4 times for Calcium, 3 times for Magnesium, 22 times for Phosphorus and 40 times for Potassium. Two granulations, C1<2 mm and C2>2 mm have been obtained by sieving the vermicompost and samples from each were taken in order to assess their microbiological characteristics.

Microbiological analyses were performed according to soil dilution method using specific solid culture media: Nutrient agar (NA) produced by Difco USA for heterotrophic aerobic bacteria and potato-dextrose agar (PDA) produced by Merck KGaA Germany for fungi (Dumitru & Manea, 2011). After 7 days incubation at dark, colonies developed in Petri plates were counted and microbial density (Total Number of Bacteria-TNB and Total Number of Fungi-TNF) was calculated and values reported to gram of dry vemicompost (d.v).

Taxonomic identification was done using morphologic criteria, by examination of microbial characteristics and measurements using a MC 5.A optic microscope. Species were identified according to the determinative manuals for heterotrophic bacteria (Bergey & Holt, 1994) and for fungi (Watanabe, 2002).

The total number of species (S) for each microbial community from C1 and C2 vermicompost granulation, as well as relative abundance (A%) and dominance of species were recorded.

The Shannon index (H′) that takes into account both the species richness and evenness (ε) was used to evaluate the microbial biodiversity in each vermicompost granulation (Mohan & Ardelean, 1993). According to Morris et al. (2014), the value of diversity index of Shannon increases with the increased number of species and when the species are evenly distributed. Similarity indices (SI) between the lists of microbial species from C1 and C2 granulations were calculated (Tiwari et al., 1994).

The global physiological activities of microflora from each vermicompost granulation were determined by substrate induced respiration method (SIR) and results expressed as mg CO_2 x100 g^{-1} vermicompost (Matei, 2011).

Circular chromatograms have been done according to the procedure recommended by Pfeiffer (1984). Vermicompost extracts have been migrated on filter paper previously impregnated with developing substances in order to obtain information concerning the biological quality of each vermicompost granulation. The image peculiarities referring to shape, dimensions, colour, texture, uniformity indicate the health, vitality, intensity of biotic activity, the level of organic matter complexity and the presence of stable humus in C1 and C2 vermicompost granulations.

RESULTS AND DISCUSSIONS

The results concerning the level of colonization of C1 and C2 vermicompost granulations with the main groups of microorganisms showed the development of high numeric effectives of heterotrophic aerobic bacteria and very high for fungi, with intense physiological activities due to the performant and complex enzymatic equipment, reflected by the high levels of soil respiration potential, as indicator of global microbial activities, in both vermicompost granulations (Table 1).

Table1. Total counts of bacteria, fungi and soil respiration from laboratory experiment with vermicompost of red worms, granulations C1 (< 2 mm) and C2 (> 2 mm)

No crt.	Experimental variant	TNB (x 10^6 viable cells x g^{-1} d.v.)	TNF (x 10^3 cfu x g^{-1} d.v.)	Soil respiration (mg CO_2 x 100 g^{-1} vermicompost)
1	C1 Vermicompost granulation	53.102	235.414	116.833
2	C2 Vermicompost granulation	106.003	261.880	123.809

Thus, the total counts of heterotrophic bacteria from C1 granulation were 53.102 x 10^6 viable cells x g^{-1} d.v. and doubled (106. 003 x 10^6 viable cells x g^{-1} d.v.) at C2 granulation.

The total counts of fungi were also higher in C2 granulation (261.880 x 10^3 cfu x g^{-1} d.v.) in comparison with the values registered in C1 granulation (235.414 x 10^3 cfu x g^{-1} d.v.). Accordingly, the global physiological activity values were more active in C2 vermicompost granulation (123.809 mg CO_2 x 100 g^{-1} vermicompost) than in C1 vermicompost granulation (116.833 mg CO_2 x 100 g^{-1} vermicompost). Results are supported by other research reporting the great increase in total numbers of bacteria and actinomycetes in the earthworm gut compared to the soil (Esakkiammal & Lakshmibai, 2013) and increased bacterial, fungal and actinomycetes counts in the 60th day of vermicomposting with *Eudrilus euginae* (Esakkiammal et al., 2015).

Taxonomic composition of bacterial microflora and species abundance in C1 vermicompost granulation is presented in Figure 1 and in C2 vermicompost granulation, in Figure 2.

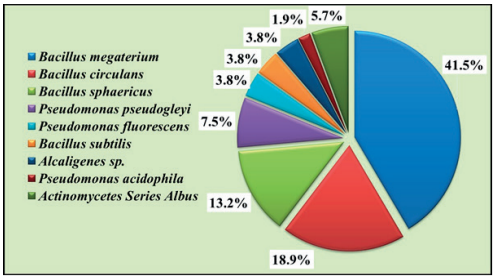


Figure1. Pie chart of percent mean relative abundance of bacterial microflora composition in C1 vermicompost granulation

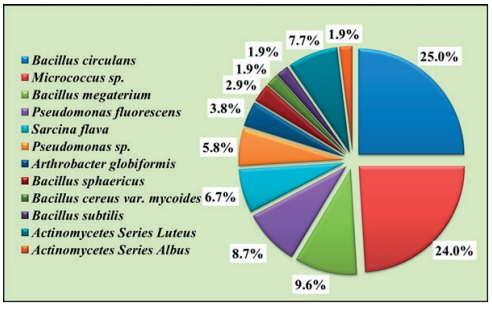


Figure 2. Pie chart of percent mean relative abundance of bacterial microflora composition in C2 vermicompost granulation

Bacterial microflora from C1 granulation consists in 4 species of bacilli (*Bacillus megaterium, B. circulans, B. sphaericus, B. subtilis*) accompanied by pseudomonads,

Alcaligenes and actinomycetes. *Bacillus megaterium*, the dominant species in C1 vermicompost granulation (A=41.5%), but also present with lower abundance at C2 granulation (A=9.6%), is known for the ability to solubilize phosphorus from inaccessible forms. Its presence indicates an advanced phase of organic matter degradation because it doesn't belong to the zymogenous (initial) microflora, its activity being characteristic to the next phase when it intervenes in degradation of more recalcitrant forms of organic matter.

Bacillus subtilis is a species belonging to zymogenous (initial) microflora, with activity oriented toward decomposition of easy degradable organic matter and more humid environments. It was identified with moderate effectives in both C1 (A=3.8%) and C2 (A=1.9%) vermicompost granulations. Its proliferation is stimulated by favourable aeration conditions. The presence of O_2 in sufficient quantities stimulates this species to degrade the organic wastes of vegetal origins during the composting process. In C1 granulation it is accompanied by *Pseudomonas acidophila* (A=1.9%) and *Pseudomonas pseudogleyi* (A=7.5%), two species adapted to live in similar (cold and humid) environment conditions. *Bacillus subtilis* is considered as a beneficial species for plants when added in biofertilizer composts/vermicomposts and present important mutual relationship with host plant. It releases different signalling molecules to improve biotic and abiotic stress tolerance or resistance, to promote plant growth, root development and to control the pathogens (Beauregard et al., 2013). Chaurasia et al. (2005) found that diffusible and volatile compounds produced by a *Bacillus subtilis* strain presented antagonistic activity against fungal pathogens by causing structural deformations. Accordingly, Boiu-Sicuia et al. (2023) reported a broad and strong antifungal effect of *B. subtilis* and other species of bacilli mediated by living cells, volatile active metabolites and cell-free supernatants against grape spoilage pathogens. Other data from literature evidenced that plant growth promotion effect was also due to volatile organic compounds produced by *Bacillus subtilis* SYST2 strain (Tahir et al., 2017).

Pseudomonas fluorescens present in both granulations with different abundance in bacteriobiome (A=3.8% in C1 and A=8.7% in C2 vermicompost granulation) belongs to zymogenous microflora acting as degrader of easily degradable organic substrates and considered one of the most important plant growth promotor bacteria from vermicompost (Shiva et al., 2013). It has a crucial role in inducing suppression of plant pathogens in rhizosphere (Matei & Matei, 2024) by producing antimicrobial metabolites (siderophores), extracellular lytic enzymes, fluorescent pigments and activation of plant defence-related mechanisms, as stated in various research from literature (Attitalla et al., 2001; Nagarajkumar et al., 2004).

Boruah & Kumar (2002) reported plant disease suppression and growth promotion by a fluorescent *Pseudomonas* strain. Another non-fluorescent species of *Pseudomonas* identified only in C2 vermicompost granulation with A=5.8%. Results of research carried out by other authors confirm the capacity of various species from genus *Pseudomonas* to produce metabolites responsible for a broad-spectrum of antifungal activity and biofertilizing properties (Kumar et al., 2005). Redouan et al. (2018) demonstrated that beneficial bacteria *Pseudomonas* spp. presented antifungal activity and were efficient agents for the control of grey and green mould. Santoyo et al. (2012) reviewed the mechanisms responsible for biocontrol and plant growth-promoting activity in bacterial species of *Bacillus* and *Pseudomonas*. Singh et al. (2013) reported the involvement of *Trichoderma harzianum* and *Pseudomonas* sp. in biocontrol of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum*). The communities of chitinase-producing bacteria vermicompost-associated showed inhibitory effects against plant pathogens *Rhizoctonia solani, Colletotrichum coccodes, Pythium ultimum, P. capsici* and *Fusarium moniliforme*, as reported in literature (Yasir et al., 2009; Veliz et al., 2017).

Previous research noticed an increase of 3.2 times of fluorescent pseudomonads numeric effectives in the variant treated with vermicompost as compared with untreated control and of approximately 4 times increase of actinomycetes counts. Relative abundance of

potential plant pathogenic species in fungal populations from variants treated with vermicompost was lower than 5%, values indicating soil suppressiveness against pathogens (Matei & Matei, 2017). The antimicrobial activity of *Pseudomonas* spp. and other bacteria and fungi was attributed to their capacity to produce siderophores, metabolites efficient in suppression of various phytopathogens and pests (Ahmed & Holmström, 2014). Other biocontrol strain (*Pseudomonas fluorescens* CHA0) presented the capability to produce secondary metabolites helping it to avoid protozoan grazing (Jousset et al., 2006).

Representatives of genus *Micrococcus* (A=24%) were co-dominant along with *Bacillus circulans* (A=25%) at C2 granulation. The spores with thick membrane produced by *B. circulans* confer to this species a high adaptability to heat and hydric stress conditions, favouring its survival. Apart of *B. circulans*, other 4 species of bacilli less abundant were identified in samples from C2 vermicompost granulation: *B. megaterium, B. sphaericus, B. cereus* var. *mycoides, B. subtilis*. Another species belonging to the family of Micrococcaceae, identified at the same granulation, was *Sarcina flava* (A=6.7%), the proliferation of this group of bacteria being connected with the presence of red worms that processed the substrate and turned it into vermicompost. The other bacteria species from microbial community from C2 granulations were represented by fluorescent and non-fluorescent *Pseudomonas* spp., *Arthrobacter globiformis* (A= 3.8%) and Actinomycetes. The last 2 taxa present a high adaptability to a wide range of nutritive substrates, from poor to rich, being favoured especially by the presence of materials previously degraded by pseudomonads from zymogenous microflora.

Actinomycetes from series Albus were present in both granulations, with higher value A= 5.7% in C1 and lower value A=1.9 in C2. Representatives of Series Luteus were identified only in C2 granulation with high relative abundance A=7.7%. Thus, actinomycetes were better represented in C2 vermicompost granulation (as number and taxonomic diversity) than in C1 granulation. Actinomycetes are active cellulase-producers for organic wastes decomposition and enrich the vermicompost in antibiotic producing agents with antimicrobial role, as resulting from literature (Augustine et al., 2008). Other authors found Proteobacteria and Actinomycetes as the dominant bacteria during earthworms' growth during vermicomposting of two types of agricultural wastes. They reported that abundance of dominant bacteria changed under the influence of earthworm activity and gut transit (Qian et al., 2024).

Taxonomic composition of fungal microflora and species abundance in C1 vermicompost granulation is presented in Figure 3 and in C2 vermicompost granulation, in Figure 4.

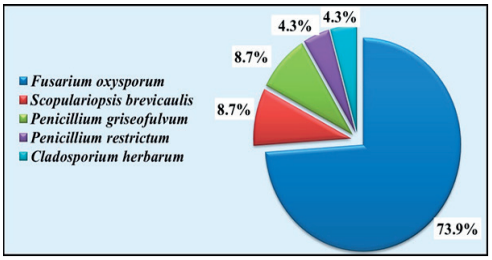


Figure3. Pie chart of percent mean relative abundance of fungal microflora composition in C1 vermicompost granulation

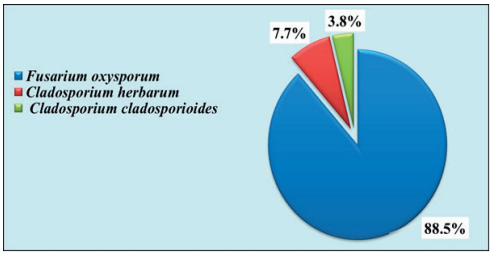


Figure4. Pie chart of percent mean relative abundance of fungal microflora composition in C2 vermicompost granulation

Fusarium oxysporum was present as dominant species in both C1(A=73.9%) and C2 (A=88.5%) vermicompost granulations, well developed and active degrader of organic materials in conditions of good aeration. Species was frequent identified in dung, various composts, including vermicomposts. It acts as antibacterial as well as antifungal agent, with role in the biocontrol of plant pathogens due to his active metabolites and stimulates plants defensive responses. The growth hormones produced by this rhizosphere species

promote plant growth and development, especially in tomato, radish, lettuce and maize.

Cladosporium herbarum, cosmopolitan species, with high ability to produce cellulolytic, amylolytic, pectinolytic enzymes and melanin, has a crucial role in binding particles of substrate in aggregates and in humification process. Species has a high affinity for rhizosphere conditions. The metabolites produced present antibacterial and antifungal activity, stimulate plant immunity, important in biocontrol and promote plant growth.

It was identified with moderate effectives in both C1 (A=4.3%) and C2 (A=7.7%) vermicompost granulations. In C2 granulation, it was accompanied by another species belonging to the same genus (*Cladosporium cladosporioides*), but less abundant (A=3.8%).

Other differential species identified only at C2 vermicompost granulation were cosmopolitan, organic waste decomposer using diversified enzymatic equipment, active producers of metabolites with antimicrobial role, belonging to genus *Penicillium*: *P.restrictum* (A=4.3%), *P. griseofulvum* (A=8.7), and *Scopulariopsis brevicaulis* (A=8.7%) with strong cellulolytic, ligninolytic, humifying capacities and specific for rhizosphere where present multiple interactions with plants.

Chaudhary et al. (2018) evidenced the role of *Penicillium* in enhancing plants resistance to abiotic stresses.

Similarity Index value calculated between the lists of bacteria identified meaned that 57.15% were species shared between the two granulations. Also, half of fungal species were common to both list from C1 and C2 vermicompost granulations (SI=50%).

Table 2 presents the comparative values of S indices (measure microbial community richness, with higher values indicating greater community richness) and Shannon indices (measure microbial community diversity, with higher values indicating greater community diversity) from C1 and C2 vermicompost granulations (Qian et al., 2024).

Thus, a rich bacteria population with S=12 species, high diversity (H'=2.104 bits) and homogeneity (ε=0.662) was found in C2 granulation. C1 granulation contained a less rich bacteriobiome (S=9 species), with H'=1.750 bits and ε=0.594.

Table 2. Species richness and Diversity indices of bacterial and fungal communities from laboratory experiment with vermicompost of red worms, granulations C1 (< 2 mm) and C2 (> 2 mm)

Vermicompost granulation	Bacterial Diversity	Fungal Diversity
C1(< 2 mm)	S=9 Shannon H'=1.750bit E(ε)=0.594	S=5 Shannon H'=0.921bit E(ε)=0.324
C2 (> 2 mm)	S=12 Shannon H'=2.104bit E(ε)=0.662	S=3 Shannon H'=0.403bit E(ε)=0.089

Fungi presented lower values of richness and biodiversity indices than bacteria. Conditions from C1 were more favourable for fungal microflora than those from C2 granulation (S=5 species, H'=0.921 bits, ε=0.324 in C1 and S=3 species, H'=0.403 bits, ε=0.089 in C2 fraction). Pfeiffer chromatograms from Figure 5 conferred supplementary information on the quality of the vermicompost granulations C1 < 2 mm and C2 > 2 mm.

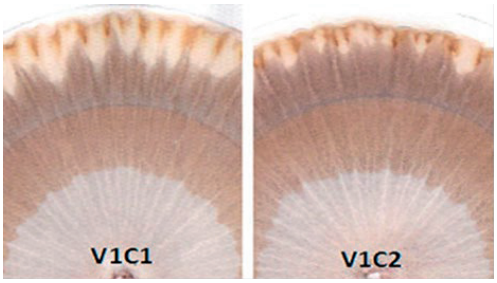


Figure 5. The aspect of sections of Pfeiffer chromatograms from C1 and C2 vermicompost granulations

The general aspect of chromatograms from the two granulations is harmonious, corresponds to a good quality of vermicompost, with distinct central, internal, intermediary and external zones present, clearly visible.

Central and internal (mineral) zones, reflecting a good structure and oxygenation during vermicomposting process, present comparable dimensions and aspect between C1 and C2 granulations.

The image continues with the integration zone of minerals, with organic matter and protein intermediary zone, the latest slightly larger in

C2 granulation. The aspect of external zone highlighted some differences concerning the significance of forms and dimensions that revealed a higher enzyme content as well as bacterial biomass, diversity and activity in C1 than in C2 vermicompost granulation. The dark coffee brown forms from the edges of external zone confirm the presence of humic substances, especially stable forms of humus in both chromatograms, but with specificity concerning their quantity and composition for each vermicompost granulation.

All the aspects of the chromatograms characterize good conditions in both vermicompost granulations, with a great richness and diversity of elements they contain. Results from recent studies (Zhang et al., 2024) attributed the higher nutritional value of vermicompost, as compared to traditional composts, to increased mineralization and humification facilitated by earthworm activity. Vermicompost improves soil characteristics (porosity, aeration, drainage, and water-holding capacity), creating favourable conditions for plant growth.

The results from our experiment with two vermicompost granulations are comparable with other research on the composition of vermicompost microflora carried out on 18 variants of organic wastes. Yampolskaya et al. (2022) reported 6 genera of micromycetes and 3 to 4 taxonomic units/variant, evidencing the importance for the synthesis of humic substances of melanin and pigments - producing genera *Cladosporium*, *Penicillium* and *Fusarium*, identified in our vermicompost granulations, too. Species from *Bacillus* and *Penicillium* genera were also reported by other authors, as part from vermicompost microflora composition. Illanjiam et al. (2019), in their study on microbial diversity of vermicompost and its efficacy on organic vegetables, identified three bacterial strains, such as: *Bacillus* sp., *Klebsiella* sp. and *Azotobacter* sp. and four fungal isolates, such as: *Aspergillus* sp., *Microsporum* sp., *Penicillium* sp. and *Trichophyton* sp. Thus, the vermicompost bacteria presented the ability to produce plant growth promoting substances as well as biosurfactants. Beneficial microbial biomass improved seed germination, seedling length and seed vitality in wheat and recommended the vermicompost obtained as effective for the improved growth of vegetable crops such as brinjal (*Solanum melongena*), ladies finger (*Abelmoschus esculentus*), chilli (*Capsicum annuum*), tomato (*Solanum lycopersicum*). Satpathy et al. (2020) reported the presence of 8 bacterial strains in vermicompost produced from a mixture of cow dung, straw, neem leaf and vegetable wastes, using earthworms (*Eisenia fetida*). All these bacteria were beneficial as they enhanced the nutrient status of vermicompost and improved the soil aeration and fertility. Species from genera *Bacillus, Pseudomonas, Micrococcus* and actinomycetes were also identified in our study on biodiversity of bacteria from C1 and C2 vermicompost granulations. Kapila et al. (2021) evaluated the microbiological quality of vermicompost prepared from 7 different types of organic wastes using *Eisenia fetida*. Authors presented the taxonomic composition of microbial community in vermicompost and the activity of hydrolytic enzymes associated with bacterial and fungal species. They identified 18 microbial taxa, 6 of them being also identified in our present experiment (phosphate solubilizers like *Bacillus* sp. and PGPBs like *Pseudomonas* sp., *Micrococcus*, actinomycetes, pigment producing actinomycetes; *Penicillium* and *Fusarium*). Microbial variation in the vermicompost was 10 to 20 times higher than in control without earthworms. The high microbial diversity present, supported significant levels of carboxy-methil-cellulase, exoglucanase, xylanase, β-glucosidase, phosphatase and urease activities. The good fertilizer value of the vermicompost recommended it as green manure and a safe method of organic waste disposal.

Other research on vermicomposting benefits recommended vermicompost containing actinomycetes and their antibiotics for obtaining chemical-free, nutritive and health protective foods in organic horticulture (Sinha et al., 2013). Previous research results (Urechescu et al., 2022) highlighted the beneficial effect of vermicompost as a culture substrate on the quality of lettuce seedlings (*Lactuca sativa*), similarly to those reported by Alper et al. (2017) on plant growth parameters and yields at the same horticultural plant. Also, other authors consider that earthworms and

vermiculture are valuable to sustainable agriculture by using them to ameliorate soils damaged by agrochemicals and other pollutants, to restore soil fertility, to improve yields and to mitigate global warming effects by soil organic carbon sequestration (Sinha et al., 2014). It was demonstrated the crucial contribution of bacterial and fungal microflora to carbon sequestration in agroecosystems (Six et al., 2006). According to Wagg et al. (2019), the high fungal-bacterial diversity and microbiome complexity have a major contribution to ecosystem functioning. The high efficiency of microbial carbon use in vermicomposting process promotes global soil carbon storage (Tao et al., 2023).

Results from present research are in concordance with those reported in a study on the possibility of granulating wet vermicompost into granules using vermiwash for easier storage and packaging. The particle size was 0.6 mm and a distribution recommended for fertilizer granules. The vermicompost NPK content increased by 48.9%, 55.4% and 75% respectively upon granulation, due to the additional fertilizer macronutrients that were supplied by the vermiwash (Manyuchi & Nyamunokora, 2014).

Other authors (Vronskis et al., 2016) obtained vermicompost pellets with 6 mm diameter at high conditioning temperatures 95^{o}C, considered a concentrated fertilizer, by reducing the volume of material more than 10 times by water removal and material thickening while extruding. The vermicompost application conferred tolerance to stress and benefits, such as: seedling vigour and plant health, attributed mainly to high concentrations of plant available macro- and micro nutrients, plant growth promoting organic acids and high microbial activity.

As in our vermicompost granulations, containing a rich microbiota with high biological activity, the beneficial effect of microorganisms in improving soil structure and forming of stable aggregates by binding capacity of fungal hyphae and bacterial exopolysaccharides was already confirmed by Gupta & Germida (2015).

Vermicompost with enriched microflora and gypsum amendments were successfully utilized for improving aggregate formation in bauxite residue (Zhu et al., 2017).

CONCLUSIONS

Both bacteria and fungi have colonized the vermicompost granulation C2 (> 2 mm) with higher counts in comparison with C1 granulation (< 2 mm).

Global microbial activity was expressed by higher values of soil respiration in C2 granulation as compared with lower values in C1 granulation.

A rich bacteria population with 12 species, high diversity (H'=2.104 bits) and homogeneity (ε=0.662) was found in C2 granulation.

C1 granulation contained a less rich and diverse bacterial community than C2 granulation, with 9 species, H'=1.750 bits and ε=0.594.

Fungi presented lower biodiversity values than bacteria.

Conditions from C1 were more favourable for fungal diversity than those from C2 granulation (H'=0,921 bits, ε=0.324 in C1 and H'=0.403 bits, ε=0.089 in C2 fraction).

Bacterial microflora from C1 granulation was dominated by bacilli, accompanied by *Pseudomonas* and actinomycetes and a mix of species (*Bacillus, Micrococcus, Pseudomonas, Arthrobacter* and actinomycetes) in C2 granulation.

In both fractions identified strong cellulolytic fungal species from genera *Fusarium, Scopulariopsis, Penicillium, Cladosporium*.

Specific paper chromatograms highlighted the good quality of the two vermicompost granulations, revealing a high microbial biomass and metabolic activity, confirmed by the presence of active enzymes and richness of humus compounds.

Further research is needed to assess the effect of the vermicompost granulation as biofertilizers for various plants cultivated in organic horticulture.

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SURVIVAL AND QUALITIES OF STEVIA SEEDLINGS DEPENDING ON THE METHOD OF REPRODUCTION

Ahmed MEHMED, Stanimir ENCHEV, Tzvetan KIKINDONOV

Agricultural Academy - Sofia, Agricultural Institute - Shumen,
3 Simeon Veliki Blvd, 9700, Shumen, Bulgaria

Corresponding author email: stanimir_en@abv.bg

Abstract

In the conditions of Bulgaria, Stevia is propagated by planting seedlings. Seedlings are produced by cuttings from stored rhizomes, from seeds and from in vitro micropropagation and rooting techniques. In the laboratory of tissue cultures of the Agricultural Institute - Shumen, methods have been developed for efficient preservation of elite clones and micropropagation. In the 2020-2023 study, the influence of genotype on the proportion of rooting in vitro was evaluated. A comparative analysis of the survival of rooted in vitro regenerants during their adaptation to external conditions was performed, compared to that of cuttings from rhizomes and propagation from seeds. Seedlings are qualified according to biometric parameters - length and mass of the stem and roots. In vitro techniques remain the safest method for preserving and propagating elite material of Stevia for Bulgaria`s conditions.

Key words*: Stevia, reproduction, micropropagation.*

INTRODUCTION

Stevia (*Stevia rebadudiana* Bertoni) is a perennial, cross-pollinated plant of the Asteraceae family. It originates from the Paraguayan and Brazilian mountains (Lewis, 1992). In recent years, there has been a heighten interest in its cultivation as a source of natural, non-caloric sweeteners. They are of the diterpene glucosides such as rebaudioside A, B, C, E and Stevioside and are up to 300 times sweeter than sucrose (Geuns, 2004).

Due to its sensitivity to low temperatures in countries with temperate climates, it can be grown as an annual, but it also can be maintained by storing the rhizomes (Lankes and Pude, 2008). Propagation by rooting cuttings from rhizomes stored in winter is widely used. An alternative method is propagation and maintenance by in vitro methods, in which the initial genotype is also preserved (Krumov et al., 1984).

In natural conditions, it reproduces by seeds. Stevia is a short-day plant and a certain light and temperature regime is required for insemination (Zaidan et al., 1980). As a cross-pollinated crop, self-incompatibility mechanisms prevent self-pollination and the production of homozygous offspring (Nakamura et al., 1985). Flowering occurs non-simultaneously and is prolonged.

As early as the 1980s, research was conducted at the Sugar Beet Institute in Shumen on the introduction and cultivation of Stevia, establishing the optimal technological conditions and developing methods for *in vitro* propagation (Vurbanov et al., 1996). With the renewed interest, Agricultural Institute - Shumen is working on the selection of new forms of Stevia. In 2017, the first Bulgarian variety with high economic qualities and adapted to the conditions of Bulgaria was recognized (Uchkunov, 2006).

Some aspects of obtaining and growing seeds in vitro and under outdoor conditions have been studied (Bozhimirov and Slavova, 2011; Kikindonov and Enchev, 2012). Under our conditions, from propagated *in vitro* and after prolonged adaptation in spring in greenhouse conditions, individual plants begin to bloom at the end of July. Mass flowering is from September until the rhizomes are harvested before the formation of autumn frosts (Bozhimirov and Slavova, 2011).

The study presents the results of the adaptation to outdoor conditions of germinated plants from seeds, rhizome cuttings and from *in vitro* regenerants to obtain seedlings for cultivation under field conditions in Bulgaria.

MATERIALS AND METHODS

The data for the study were obtained from research in the period 2021-2023 at Agricultural Institute Shumen, Bulgaria.

The study used selection material from the gene pool of Agricultural Institute - Shumen. The sources are from Japan, which are smaller-leaved, densely overgrown, and from America with larger and thicker leaf petioles. After many years of work on individual, family and clonal selection and adaptation to our conditions, elite clones have been formed, as well as origins - a product of somaclonal and gametoclonal variation in *in vitro* culture of meristems, embryos, seeds.

Perennial rhizomes from 15 to 30 elite clones, annual rhizomes from clones grown from seedlings in the previous year and new seedlings from *in vitro* micropropagation of supported clones, cuttings from rhizomes and from seeds are stored and planted annually.

In adaptation rooms with controlled temperature, studies are carried out on seed germination, adaptation of rooted *in vitro* regenerants to external conditions, rooting of rhizome cuttings and young plants, picking and growing seedlings suitable for planting in field conditions.

The cleaned seeds are sown in a soil substrate of peat, sand and perlite in a ratio of 4/1/1 in pots, by evenly distributing them on the surface with pressing without backfilling. A moist chamber is provided with a polyethylene cover tightly for 4-6 days and with gradual opening up to the 14th day.

Under the same conditions, the rooted in vitro regenerants and cuttings with 3-5 leaf nodes from the tips of the rhizome buds, cleaned from the nutrient medium, are planted. For the first 20 days until the development of the root system, they can be planted more densely, since some of them do not develop roots or rot.

To obtain suitable seedlings, 45 to 60 days of cultivation are required, and for seed offspring this period is extended to 75 days. Then they are taken outdoors for 14-20 days for hardening before planting in field conditions.



Figure 1. Stevia reproduction

RESULTS AND DISCUSSIONS

Adaptation of germinated plants from seeds to external conditions for obtaining seedlings for field cultivation

The trend for a longer and warmer autumn in Bulgaria in recent years has made it possible to cover the requirements of the Stevia plant for combined temperature and short day at the end of the growing season, to obtain viable seeds. In our study, whole plants are harvested from the field at the end of the season and dried. Then the seeds are collected by threshing, without separating the empty from the full seeds. The seeds collected by threshing of dried whole plants have an average germination rate of

21.8%, with a coefficient of variation of 22.6%. This allows for a more widespread use of seeds for propagation in practice.

In order to increase the volume of Stevia seedlings, the obtained seeds from 2023 were sown in series every 15-20 days in pots with a soil mixture of 100 grams of origin. On the 14th day, the germinated seeds were counted. After this period, some of the germinated plants did not survive and 45-50 days after germination, the surviving plants were counted and dived to obtain adapted seedlings for planting in the field (Table 1).

Previous studies have established the mass levels of 1000 seeds of 400 mg or 250 seeds are sown with 100 g. This significantly facilitates the technology for accelerated propagation.

The germination in the soil mixture and the survival of seeds from the 2023 harvest is relatively high, with a high degree of variation depending on both the genotype and the type of seedlings. Most viable are seeds from one-year-old seedlings. The genotype is a determining factor for seed reproduction within a single harvest. For Bulgaria, the specific agroclimatic conditions of the year are the limiting factor.

Table 1. Seed germination and survival to external conditions for obtaining Stevia seedlings, 2023

Origin of donor plants	One-year-old seedlings		Two-year-old rhizomes		Perennial rhizomes	
Origin	Sprouted seeds	Survival rate	Sprouted seeds	Survival rate	Sprouted seeds	Survival rate
	amount	%	amount	%	amount	%
1	9	11.1	10	90.0	34	94.1
2	127	78.0	9	66.7	95	84.2
3	6	16.7	4	25.0	5	40.0
4	123	69.9	85	63.5	6	33.3
5	92	91.3	89	78.7	11	9.1
6	80	80.0	66	81.8	6	33.3
7	110	72.7	123	79.7	74	67.6
8	61	90.2	65	86.2	77	80.5
9	95	86.3	91	87.9	93	36.6
10	124	95.2	5	20.0	34	38.2
x	82.7	69.1	54.7	67.9	43.5	51.7
vc	54.1	43.7	80.4	37.4	86.3	53.8
s x±x	14.1	9.57	13.9	8.05	11.8	8.80
p%	17.1	13.8	25.4	11.8	27.2	17.0

Rooting cuttings from rhizomes

To establish the parameters for effective rooting of cuttings in 2022, 7 genotypes were selected, with plants from each being planted on two different dates - one on 25.03 and the other on 25.05. The aim was to determine whether the planting dates had any effect on the rooting percentage of the plants. From Table 2 it can be clearly seen that the percentage of rooted plants in March was 79.76%, while the percentage in May was 67.38%. The only deviation from the average values was observed in two of the origins A10 and E7, which is most likely due to genetic factors.

Table 2. Rooting cuttings from winter-stored rhizomes of elite Stevia plants, 2022

Variants	25.03			25.05			Total	Total rooted
Genotypes	Studied	Rooted	Rooted %	Studied	Rooted	Rooted %	amount	%
75 ШЗ	48	38	79.2	48	26	54.2	96	66.67
210/9	30	27	90.0	30	18	60.0	60	75.00
214/2	30	28	93.3	30	26	86.7	60	90.00
7 E	30	22	73.3	30	24	80.0	60	76.67
9 E	30	23	76.7	30	11	36.7	60	56.67
A 10	48	26	54.2	48	38	79.2	96	66.67
CP 28	48	44	91.7	48	36	75.0	96	83.33
X	**37.7**	**29.**	**79.7**	**37.7**	**25.5**	**67.3**	**75.4**	**73.5**
Sx	**3.64**	**3.09**	**5.20**	**3.64**	**3.57**	**6.73**	**7.27**	**4.25**
CV	**25.5**	**27.**	**17.2**	**25.5**	**36.9**	**26.4**	**25.5**	**15.2**
P %	**9.64**	**10.4**	**6.52**	**9.64**	**13.9**	**9.98**	**9.64**	**5.77**

In 2023, this experiment was expanded and a total of 15 origins were used to monitor effective rooting of cuttings, with the cuttings being planted in three groups in different months – January, February and March, preceding the dates of the previous study. In addition to the 7 origins planted in the first experiment, 8 more origins were added. From Table 3, it can be clearly seen that the percentage of rooted plants in January is 98.54%, decreasing to 81.96% in the following month and to 72.95% in March. Once again, a deviation was observed in some of the origins – 7E, 9E and A10, where in February there was less or the same percentage of rooted plants compared to March. This confirms the claim that hereditary factors are important for the rooting of plants. It is also impressive that the percentage of rooted plants in March 2022 and 2023 is approximately the same for the origins used in both experiments. From the conducted observations, it can be concluded that for the most successful rooting of the plants, the cuttings should be planted as early as possible in the year.

Table 3. Rooting cuttings from winter-stored rhizomes of elite Stevia plants, 2023

Origin	26-30.01		28-30.02		26-30.03		Total amount	Total rooted %
	Studied	Rooted %	Studied	Rooted %	Studied	Rooted %		
3	30	100.0	45	97.8	20	85.0	95	95.79
4	28	100.0	35	60.0	25	48.0	88	69.32
5	18	100.0	30	80.0	15	60.0	63	80.95
1-1	25	92.0	30	86.7	15	66.7	70	84.29
10 ШЗ	31	100.0	40	85.0	25	80.0	96	88.54
75 ШЗ	20	95.0	15	93.3	20	80.0	55	89.09
203/2	15	100.0	15	86.7	15	73.3	45	86.67
210/9	36	97.2	45	91.1	22	77.3	103	90.29
214/2	43	97.7	15	93.3	15	86.7	73	94.52
7 E	11	100.0	15	73.3	15	80.0	41	82.93
9 E	17	100.0	15	60.0	15	73.3	47	78.72
A 10	40	100.0	45	62.2	25	60.0	110	75.45
CP 27	26	96.2	30	76.7	15	60.0	71	80.28
CP 28	15	100.0	30	90.0	20	80.0	65	89.23
CP 162	13	100.0	30	93.3	25	84.0	68	91.18
x	**24.5**	**98.5**	**29.0**	**81.9**	**19.1**	**72.9**	**72.6**	**85.1**
Sx	**2.60**	**.64**	**3.02**	**3.31**	**1.13**	**2.96**	**5.57**	**1.88**
CV	**40.9**	**2.50**	**40.2**	**15.6**	**22.8**	**15.7**	**29.6**	**8.55**
P %	**10.5**	**0.64**	**10.4**	**4.04**	**5.90**	**4.06**	**7.67**	**2.21**

Adaptation of rooted in vitro regenerants

Between 2021 and 2023, an assessment of the rooting under outdoor conditions of regenerants obtained from in vitro cultures was carried out (Table 4). Fifteen origins were used, and the percentage of rooted plants was measured for each origin. The results are presented by year as well as in total for the three years. An increase in rooting was observed for each subsequent year, which may be due to environmental factors. On average, 236 regenerants were used for each of the origins, with the total number for individual genotypes varying between 172 and 338 individuals. The average rooting percentage for the three years is 87.8%. It is interesting to note that for some of the genotypes (20% of the genotypes) the rooting is 100%. As 60% of the origins achieve rooting, in total for all three years, more than 90%.

Table 4. Rooting of *in vitro* regenerants in outdoor conditions, 2021-2023

Year	2021		2022		2023		Total for the period	
Genotypes	Total amount	With root %	Total amount	With root %	Total amount	With root %	Total amount	With root %
3	120	100.0	90	100.0	100	86.0	310	95.5
4	74	94.6	155	54.8	100	90.0	329	74.5
5	105	36.2	57	96.5	120	95.0	282	73.4
1-1	100	37.0	120	90.0	100	88.0	320	72.8
10 ШЗ	100	100.0	100	85.0	75	93.3	275	92.7
75 ШЗ	120	89.2	70	100.0	185	100.0	375	96.5
203/2	105	100.0	90	85.6	100	90.0	295	92.2
210/9	100	67.0	100	91.0	100	85.0	300	81.0
214/2	30	86.7	145	97.9	95	87.4	270	93.0
7 E	143	93.0	120	90.8	80	95.0	343	92.7
9 E	174	89.1	120	85.8	100	89.0	394	88.1
A 10	150	59.3	150	90.0	95	92.6	395	79.0
CP 27	120	70.8	70	100.0	105	100.0	295	88.1
CP 28	60	100.0	100	96.0	112	98.2	272	97.8
CP 162	75	74.7	175	92.6	163	96.3	413	90.8
x		**79.8**		**90.4**		**92.3**	**324.5**	**87.2**
Sx		**5.64**		**2.88**		**1.28**	**12.5**	**2.25**
CV		**27.3**		**12.3**		**5.35**	**14.9**	**10.0**
P %		**7.06**		**3.19**		**1.38**	**3.87**	**2.58**

Evaluation of adapted seedlings from in vitro regenerants and from rhizome cuttings

Table 5 displays the evaluation of adapted seedlings from *in vitro* regenerants and from rhizome cuttings. From the data on the evaluation parameters of the two groups it is noticeable that, plants obtained from in vitro regenerants have higher indicators than those obtained from cuttings. Parameters such as stem height, number of leaves, root length, as well as the weight of dry matter (root, leaf, stem) were compared. The observed difference in terms of height, number of leaves and root is significant and is in favor of plants obtained from *in vitro* regenerants. The total dry matter of plants obtained from cuttings is also less than that of plants obtained from *in vitro* regenerants. When analyzing the different parts of the plants, it was found that the weight of dried roots and stems in plants obtained from in vitro is less compared to the same weight of plants obtained from cuttings. Conversely, the dry weight of leaves in plants obtained from cuttings is greater than that of leaves in *in vitro* regenerants. This applies not only to the average leaf weight, but also to each plant compared individually.

Table 5. Evaluation of adapted seedlings obtained from *in vitro* regenerants and from rhizome cuttings, 2023

Variants	Stem height, cm	Amount of leaves	Root lenght	Weight (in g) of:			
				Root	Leaves	Stem	Total dry mass
Cuttings							
1.	13.5	10.0	6.0	0.48	0.44	0.28	0.24
2.	11.0	13.1	5.2	0.23	0.34	0.13	0.14
3.	10.5	10.7	3.5	0.17	0.16	0.27	0.12
4.	10.9	10.0	9.9	0.47	0.29	0.14	0.18
5.	14.5	12.0	7.0	1.04	0.44	0.21	0.34
6.	21.0	12.3	6.1	0.40	0.63	0.30	0.26
7.	15.2	12.0	5.1	0.48	0.44	0.17	0.22
8.	16.2	10.6	5.4	0.41	0.32	0.18	0.13
9.	14.1	10.0	9.4	0.47	0.38	0.17	0.21
10.	11.4	10.6	7.4	0.41	0.34	0.15	0.18
11.	17.2	14.0	8.4	0.33	0.59	0.28	0.24
12.	8.0	10.0	5.6	0.19	0.23	0.18	0.18
Average	**13.6**	**11.3**	**6.6**	**0.42**	**0.38**	**0.20**	**0.20**

Regenerants							
1.	23.5	16.0	12.1	0.40	0.25	0.30	0.26
2.	19.5	16.3	9.5	0.49	0.26	0.43	0.31
3.	21.0	14.0	11.0	0.61	0.30	0.48	0.38
4.	23.4	18.0	13.0	1.07	0.25	0.47	0.48
5.	18.2	16.3	11.0	0.60	0.27	0.38	0.34
6.	18.0	16.0	9.8	0.67	0.23	0.41	0.35
7.	17.0	12.0	9.0	0.77	0.22	0.42	0.38
8.	16.7	12.3	8.5	0.38	0.23	0.31	0.25
9.	17.2	14.0	11.0	0.90	0.30	0.48	0.45
10.	18.0	14.0	13.0	0.74	0.23	0.52	0.40
11.	17.5	16.3	15.0	0.91	0.31	0.52	0.49
12.	12.5	12.0	11.0	0.28	0.12	0.29	0.49
Average	**18.5**	**14.8**	**11.2**	**0.65**	**0.24**	**0.42**	**0.35**

CONCLUSIONS

The assessment of adaptation to external conditions and the development of seedlings under field conditions allows us to draw certain conclusions important for the practical implementation of Stevia.

Germination in soil mixture and seed survival is relatively high, with a high degree of variation depending on both the genotype and the duration of use of donor plants. The most viable are seeds from one-year-old seedlings. The genotype is a determining factor for seed reproduction within one harvest.

The average rooting rate of in vitro regenerants under external conditions for the three years in total is 87.8%. It is interesting to note that for 20% of genotypes, rooting is 100%, and 60% of origins achieve rooting of more than 90%.

Research has shown that the timing of cuttings is critical for successful rooting of plants. The highest rooting rates are observed for cuttings planted early in the year, with January showing the best results followed by February and March. This highlights the importance of early planting for optimal rooting. Hereditary factors also play a role, with some genotypes showing deviations from the average values.

Evaluation of rooting of regenerants from in vitro cultures indicates that these plants have better morphological and economic indicators compared to plants obtained from cuttings.

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THE EVOLUTION OF CLIMATE CHANGE IN THE WESTERN REGION OF ROMANIA AND THEIR INFLUENCE ON CROPS

Vlad Dragoslav MIRCOV, Carmen Claudia DURĂU, Veronica SĂRĂȚEANU, Casiana MIHUȚ, Daniela Nicoleta SCEDEI, Anișoara DUMA COPCEA, Marius BOLDEA

University of Life Sciences "King Mihai I" from Timișoara, 300645, 119 Calea Aradului, Timişoara, Romania, Phone: +40256277001, Fax: +40256200296

Corresponding author email: casiana_mihut@usvt.ro

Abstract

In this paper, we have made an interpretation of how climate changes and certain risk factors have influenced the evolution of the climate in the western part of Romania. With the help of meteorological data provided by the representative stations in this part of the country, the influence of abiotic factors was analyzed for different study intervals. The first part of the paper presents a period with a normal multiannual evolution of the thermal regime, the pluviometric regime and extreme phenomena, and in the second part, the years with significant deviations are interpreted. The analysis of meteorological parameters was carried out for the representative stations in the western and northwestern areas of the country, stations belonging to the Banat Crișana Regional Meteorological Center, respectively those in the counties of Timiș, Arad and Bihor. The results obtained led to the formulation of some conclusions regarding the influence that these climate changes have on the crops in the analyzed counties.

Key words*: climate change, crops, meteorological stations, pluviometric regime, risk factors.*

INTRODUCTION

Of all the terrestrial geospheres, the atmosphere is the most unstable, the most fragile and vulnerable, being in a precarious balance. It is constantly exposed to meteorological and climatic imbalances, which, in turn, trigger other environmental disturbances in the chain. Since humanity has a direct interest in maintaining this balance (Bogdan, 2006).

Global warming is causing changes in different regions of the world that include increased frequency and severity of storms, hurricanes, floods, landslides, extreme heat or cold, droughts, water shortages, forest fires and other disasters(https://www.mmediu.ro/categorie/schimbari-climatice/1).

At the European level, the main objectives regarding climate change are presented in the Convention on Climate Change (UNFCCC) and in the framework of the Kyoto Protocol (European Environment Agency, 2004). Chiper (2015) states that "the effects of climate change are negative elements for agriculture" because they propagate in agricultural territories.

South-west Romania, like the entire country, due to its geographical position in the temperate zone, is exposed to a wide range of risk meteorological and climatic phenomena with the potential to occur throughout the year (Cristea, 2022; Drăghici, 1988; Durău, 2024; Mircov et al., 2024; Mihuț et al., 2024).

Along with Romania's geographical position in relation to the main pressure centers, the characteristics of the relief create great differences in terms of the genesis and distribution of atmospheric precipitation, both throughout the country and above Banat (Mihuț et al., 2024; Pop, 1988).

Banat is located in a very varied geographical setting, which includes relief forms whose altitude increases from west to east, like an amphitheater (Nichita et al., 2010).

For a correct assessment of the climatic conditions specific to a certain territory, a series of information related to climate, hydrology, geology, vegetation and soil properties is needed (Mihut et al., 2024).

The present work aims to identify some meteorological indicators that most accurately express the extreme nature of the manifestations of atmospheric phenomena and for this we analyzed a ten-year interval, an interval in which certain climatic changes were highlighted.

MATERIALS AND METHODS

In this paper, we analyzed the deviations of meteorological risk factors recorded at meteorological stations located in the Western part of Romania, stations belonging to the Banat-Crișana Regional Meteorological Center. We highlighted the data obtained from the stations in the counties of Arad, Bihor, Caraș-Severin and Timiș (Mircov et al., 2024).
The thermal regime, the pluviometric regime and certain aspects of the wind regime, a regime specific to the area in the South-West of Romania, the Coșava wind, were highlighted. A more detailed analysis of certain phenomena was made in order to capture exceptions, special situations or more significant meteorological elements.

RESULTS AND DISCUSSIONS

The evolution of risk aspects in these years was presented for certain representative stations, for which we took into account the specific risk parameters. For the Banat area, the warmest month of the year is July, with average temperatures of 21.5ºC and the coldest is January, when the average temperature reaches 1.5-2ºC. The variation of the annual average temperature presents approximately the same aspect at all stations, the amplitude and the values encountered differ (Munteanu, 2001).
The highest values in the summer months are found at the southern stations with average temperatures of over 22ºC at Moldova Veche and Banloc. The lowest average temperatures are recorded, except for the mountain stations, in the Bozovici depression area, where the temperature does not reach 20ºC. January is the coldest month with averages between 0ºC and 3ºC, with the lowest value in the Bozovici Depression and in the Mureș Corridor at the Vărădia de Mureș station, due to an inversion, and the highest in the south, at Oravița and Moldova Veche.
The mountain area has the lowest temperatures, which is normal due to the altitude, the average in January reaching -8ºC at Țarcu. For the analyzed interval, it can be observed that, in Timișoara, in July the average values exceeded 22ºC, the highest value being recorded in 2021, which was 25.7ºC, and in 2022, when 25.2ºC was recorded (Figure 1).

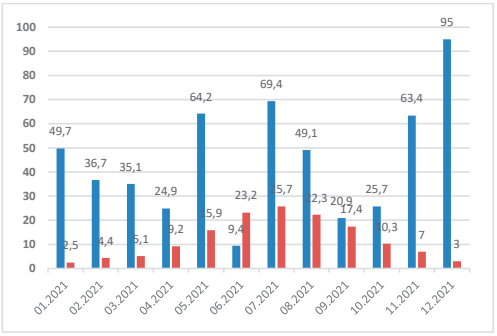


Figure 1. Thermal and rainfall regime recorded in Timisoara, in 2021 (The blue color - represents the average annual precipitation, in l/m^2; red color - represents the average temperature in °C)

If we refer to the 2015-2025 period, we can say that for the western area, the lowest monthly temperature values were recorded in July 2016, 22.9ºC, and in 2018, when we had 22.6ºC (Figure 2).



Figure 2. Thermal and rainfall regime recorded in Timisoara in 2018 (The blue color - represents the average annual precipitation, in l/m^2; red color - represents the average temperature in °C)

Summer is dominated by formations related to the Azoric anticyclone and the Mediterranean cyclones, starting early, sometimes even in May, and lasting until September. The average temperature of the hottest month, July, varies between 21-22ºC in the Oravița - Moldova Veche area and 8ºC on the highest peaks. There are hot days, with maximums exceeding 35ºC and tropical nights with minimums over 20ºC (Moldovan, 2003).
For the analyzed interval, it can be observed that, in Timișoara, in July the average values

exceeded 22ºC, the highest value being recorded in 2021, this being 25.7ºC (Data provided by CMR Banat Crișana). This year, Timișoara recorded 16 hot days in the summer months. Compared to 2021, in 2018 there were 11 hot days during the summer, and in 2022, 12 days.

From the point of view of the rainfall regime, it can be observed that the least precipitation recorded in Timișoara was recorded in October, November, December of 2019, the period in which it rained only 57 l/m^2, and this period continued in the spring of 2020, the year in which in April there were only 7 l/m^2, and in May 29 l/m^2, which led to certain decreases in sunflower and corn production (Barbu et al., 2003).

In Caraș-Severin County, in the area of Moldova Veche, two summer months of 2020 were noted for excessive rainfall, and we must particularly highlight the days of June 22, June 23, 2020, days characterized by strong instability, with convective rains over relatively extensive areas, locally significant in quantity. Regarding the average annual temperature of the area, we mention that it is higher (Figures 3 and 4).

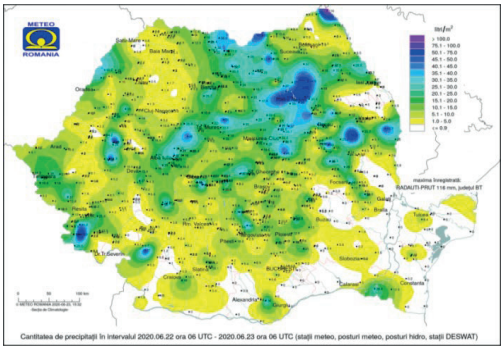


Figure 3. Amount of precipitation on 22/23 June 2020 (Synoptic code, Institute of Meteorology and Hydrology, Bucharest)

Due to the favorable climatic conditions in the fall of 2020 and the first months of 2021, in certain regions of Caraș-Severin County, in the Moldova Nouă area, high wheat productions were obtained in 2021, a quantity of 8.10 t/ha. It is worth noting the excess rainfall that was recorded in the first two months of 2021, in Oravița 157 l/m^2, while in Moldova Nouă, 128 l/m^2 accumulated in the two months. In January 2021, the most rain fell on January 2, a quantity of 18.9 l/m^2, in Moldova Nouă, and also on January 2, in Oravița 20.6 l/m^2 accumulated. In February, in Moldova Nouă it rained 10.0 l/m^2, on February 26, while in Oravița, on February 17, 26.0 l/m^2 accumulated. Regarding the corn crop, the lowest quantity was obtained in 2022, more precisely 4500 kg/ha, due to the deficient rainfall regime in all regions, and in 2021 there were values between 7000-8000 kg/ha, while in 2020 10.000 kg/ha were obtained.

The average annual isotherm of 110 delimits the Danube corridor. Due to these assessments, we signal the presence of biocenoses specific to the area. Thus, particularly intense air currents, of the bora type, locally called Coșava, develop. The Coșava wind has a turbulent character, and, according to the wind rose, the predominant directions are those from the east and west (Ghibedea, Băcanu & Lucia, 1982). The speed can reach up to 85 km/h, with gusts of up to about 135 km/h and 140 km/h, with an average duration of up to one day, sometimes even two days.

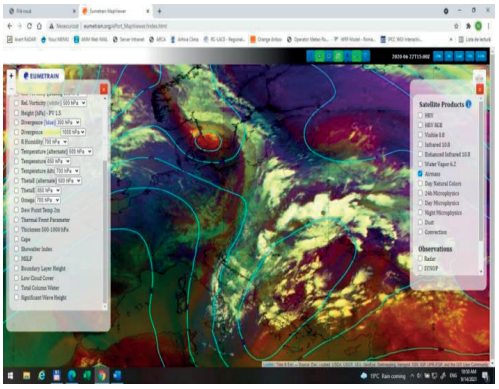


Figure 4. Satellite image (INFRARED-Air masses superimposed over the geroptential at 500 mb on 22.06.2020 (www.theweatherprediction.com/blocking/)

In the western part of the country, the highest amounts of precipitation were in Caraș-Severin, with 52 l/m^2 in Sasca, 42 l/m^2 in Oravița and 30 l/m^2 in Moldova Veche.

Starting with June 2020, when 87 l/m^2 were recorded, and until October, the rainfall regime reached 356 l/m^2, while in two months, July and October, it rained over 100 l/m^2 in Timisoara (Figure 5).

If we take into account the values obtained from the meteorological stations in Arad and

Chișineu Criș, we can say that 2020 was the rainiest year, with two consecutive months in which the amount of precipitation exceeded 100 l/m^2. In June and July, 255 l/m^2 of rain fell, more precisely, in June 145 l/m^2, and in July 108.7 l/m^2 (Figure 5).

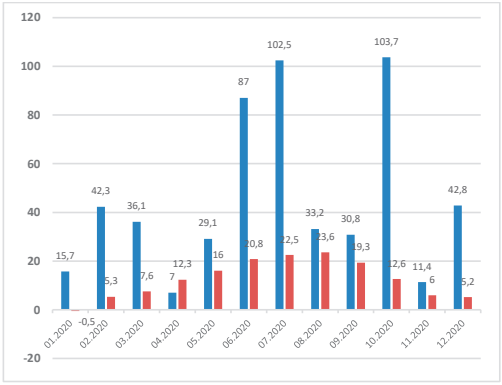


Figure 5. Thermal and rainfall regime recorded in Timisoara, in 2020 (The blue color - represents the average annual precipitation, in l/m^2; red color - represents the average temperature in °C)

In 2022, in September, we had a record excess of 148.9 l/m^2, but also a dry month during the summer, in June, when only 3.5 l/m^2 were recorded. From a thermal point of view, we can say that in 2020 the thermal regime was the coolest, with an average temperature in January of -1.3 degrees C, and the average in August was 22.3 degrees C (Data provided by CMR Banat Crișana).

At the Știei meteorological station, in July 2022 a monthly average of 22.7°C was recorded, and in 2023, the value of 22.2°C, in August. If we also analyze the last years, we can say that during this period the pluviometric regime was deficient, while the thermal values were very high. The weather was warm in most of the country in the last two years, and in the western region there was a heat wave in extensive areas. The temperature-humidity index, the ITU index, reached and exceeded the critical threshold of 80 units in Banat and Oltenia, in most of Muntenia and Crișana, and locally in Dobrogea, southwestern Transylvania and southern Moldova.

In 2023, it rained sufficiently in the first five months, the hectoliter weight of wheat decreased and that is why we had a lower production compared to 2022. In the first four months, in certain localities in the vicinity of the city of Arad, over 400 l/m^2 were recorded, which was a year with excess rainfall for this period, in the last ten years. There were days when it rained and 30-35 l/m^2, in five days, in March and over 30 l/m^2 were recorded in 4 days in April.

Due to the favorable climatic conditions, both in terms of temperature and rainfall, specific to the Chișinău Criș and Arad areas, in the fall of 2020 and the first months of 2021, the highest corn production was achieved, an amount of 8000 to 9000 kg/ha, to corn culture. It is worth noting the excess rainfall recorded in the first two months of 2021, in Chișinău Criș 137 l/m^2 were reported, while in the first four months of 2023 it rained 410 l/m^2. This favorable rainfall regime will give a production of approximately 9100 kg/ha, in the fall of this year. Also this year, a quantity of 5200 to 6000 kg/ha was obtained for wheat, while the production for rapeseed was 3480 kg/ha.

On July 21, 2023, a strong storm was recorded in Arad County. It rained 39 l/m^2, but this rain was followed by the entry into the region of a front accompanied by clouds with high vertical extension, of the Cumulonimbus type, clouds accompanied by heavy hail. This phenomenon compromised certain productions, especially in the sunflower crop, where productions between 1400 kg/ha and 1500 kg/ha were obtained.

We must also note a situation recorded at the end of 2023, when it was particularly hot throughout the country, in the last decade of the year. In the western region of our country, on 26.12.2023 - 27.12.2023, the following daily records were recorded, values that represent the highest daily values in the entire series of observations recorded on this calendar date at certain stations, such as: Banloc (15.6 compared to 14.2°C), Bozovici (13.4 compared to 12°C), Jimbolia (14.3 compared to 13.5°C), Lugoj (15 compared to 14.7°C), Moldova Veche (15.8 compared to 14.5°C), Sânnicolau Mare (14.4 compared to 13.6°C), Vf. Cuntu (9.8 compared to 9.4°C), In mid-August 2024, between 12.08-14.08, in certain stations in Timiș and Arad counties, thermal minimums between 20°C and 27°C were reported, in Șiria. Towards noon, in Moldova Veche there were 38.3°C, compared to the previous record, the value of 37.8°C. At the mountain station in

Caraș-Severin county, in Țarcu, the record of 15.2°C was reported, while in Timișoara, the minimum was 21.6°C.

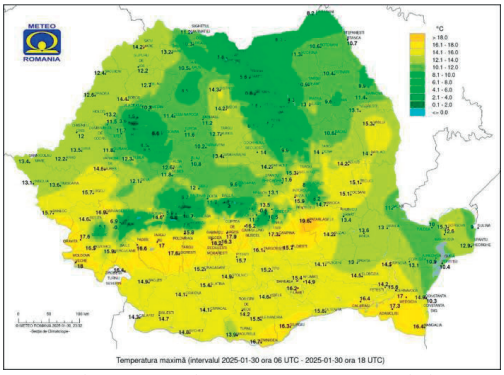


Figure 6. Maximum temperature recorded on 30.01.2025, at 6 pm
(Synoptic maps, CMR Banat Crișana, Timișoara)

The minimum temperature was equal to the highest value in the entire series of observations at this date. The last decade of 2025 was also characterized by particularly warm weather for the end of January, so that the daytime thermal values were up to 12-13°C higher than the multiannual averages of the period. On 30.01.2025-31.01.2025, 16.9°C were reported in Caransebeș, compared to 15°C, in Moldova Veche 18°C, compared to the record of 16.5°C, while in Oravița it was 17.6°C, compared to 16.2°C (Figure 6).

CONCLUSIONS

Southwestern România, like the entire country, due to its geographical position in the temperate zone, is exposed to a wide range of risk meteorological and climatic phenomena with the potential to occur throughout the year, the evolution of the thermal and pluviometric regimes in the western area being factors that decisively influence agriculture in the region.

Following the studies carried out in the Western and South Western part of Romania, we can affirm the fact that, due to the favorable rainfall conditions in 2020 and the first part of 2021, the productions obtained in the corn crop were clearly superior compared to the other years studied.

Another favorable year for the corn and wheat crop was 2023, the year in which the amount of precipitation was over 400 l/m^2, the amount recorded in the first four months in certain areas of the West.

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PORTULACA GENOTYPES BREEDED AND BIOBANKED AT PLANT GENETIC RESOURCES BANK BUZĂU

Geanina NEGOŞANU[1], Costel VÎNĂTORU[1], Bianca MUŞAT[1], Camelia BRATU[1], Matilda POPESCU[1], Adrian PETICILĂ[2]

[1]Plant Genetic Resources Bank, 56 Nicolae Bălcescu Blvd, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: geanina.neacsu11@gmail.com

Abstract

Portulaca grandiflora and P. oleracea were introduced into breeding studies at Plant Genetic Resources Bank (PGRB) Buzău. The germplasm collection of P. grandiflora contains several genotypes highlighted by a varied color palette, while for P. oleracea several genotypes have been developed that can be cultivated and have great production potential. Among them, the cultivar G2 stands out, demonstrating distinct phenotypic expression which will be presented in this article. Following biometric measurements and phenological observations, average values were recorded with a height of 68 cm, a diameter of 82 cm, and a plant weight of 1570 g, with the note that the production potential could be doubled if staggered harvesting is practiced. The results recommend Portulaca as a viable food alternative with valuable nutritional and medicinal properties, especially in the context of climate change.

Key words*: germplasm collection, production potential, phenotype, food alternative, climate change.*

INTRODUCTION

The *Portulacaceae* family counts more than 100 species worldwide (Ocampo, 2013). The best known in Romania are *Portulaca oleracea* L. or Purslane and *Portulaca grandiflora* P. or Stone flower, the first one being considered an edible wild plant and one of the three most commonly reported weeds worldwide (Carrascosa et al., 2023). Known as a noxious weed that affects conventional crops, it is also traditionally eaten in Spain, Greece, Italy, Turkey, USA, and China (Feng et al., 2015). The most appreciated parts of purslane are fresh leaves and stems, with a distinctive sour taste (Petropoulos et al., 2019), purslane being mainly used in fresh green salads, but they also can be pickled or cooked (Takergari et al., 2013). The unanimous appreciation is due to an exceptional nutritional content, *P. oleracea* being rich in omega-3 fatty acids, antioxidants such as vitamins A, C, E and B, minerals such as potassium, calcium, magnesium, especially when plants are grown under stress conditions where higher concentrations of beneficial compounds are found (Montoya et al., 2023). These compounds are associated with the pharmacological properties of the species, such as its antioxidant, anti-inflammatory, antidiabetic, anti-obesity and hepatoprotective potential among others, which have led some to consider purslane a "food of future". A halophytic species, perfectly adapted to water deficit, high salinity and temperatures, it can be integrated with minimal crop costs in order to recover and improve soil quality (Carrascosa et al., 2023).

Perceived in Romania as a harmful weed to crops, *P. oleracea* occurs among vegetable or flowers seedlings, in orchards but also in wasteland, growing solitary or forming a green carpet covering the soil (Figure 1).



a) b)

Figure 1. *P. oleracea* grown in: a) basil seedling; b) on asphalt

1It vegetates in the most harsh places and when it is uprooted and left in the ground purslane doesn't die, but, on the contrary, it has the ability to emit adventitious roots on all its vegetative organs at soil contact, resuming its vegetation cycle and thus speeding up the rate of maturation and self-sowing in the ground. At PGRB Buzau, starting with 2021, *P. oleracea* and *P. grandiflora* species have been taken under study, by introducing them in breeding programs. Thus, several genotypes with distinct phenotypic expressivity were obtained, both in *P. grandiflora* for ornamental purposes and cultivable varieties, in *P. oleracea*, with high yield potential and a lifespan between 3 and 4 months. This article describes the *P. oleracea* G2 genotype, which was characterized by valuable biometric and phenological traits.

MATERIALS AND METHODS

The research works were carried out at PGRB Buzau (45°09´N, 25°5´E, 95 m) located in SE Romania, characterized by continental climate, steppe characteristic vegetation and leached chernozem soil.

The period analysed in this study was 2024. The genetic resources of *P. oleracea* come from the PGRB Buzau seed collection. Three cultivated genotypes were analysed: G1, G2 and G3 (Figure 2).



a) b)

Figure 2. *Portulaca oleracea* plants: a) cultivated genotype; b) wild purslane

The sowing was done in the first decade of March in protected spaces. Seeds were placed in 70-cell black plastic trays filled with commercial peat under nursery conditions. Seedlings were transplanted after 45 days, on 05.05.2023, at the stage of 6-8 leaves and 7-8 cm in height. No fertilizers, insecticides or other agrochemicals were used during the seedling growth.

The breeding methods used were repeated individual selection followed by negative mass selection. Phenological observations and biometric measurements were carried out according to the UPOV and IPGRI data sheets. Weighing operations were performed at PGRB Buzau laboratory on green plants and the device used was the two-decimal precision balance Kern 572-33. Leaf chlorophylls was assessed and the results were expressed in CCI units, using a CCM-200 plus, chlorophyll content meter (Figure 3).



a) b)

Figure 3. Laboratory instruments: a) CCM-200 plus, chlorophyll content meter; b) two-decimal precision balance Kern

Data were statistically analysed by the IBM SPSS v29.0. (SPSS Inc., Chicago, IL, USA) program. Prior to the analysis of variance (ANOVA), data were checked for normality. Then raw data for each genotype were subjected to one-way ANOVA, and when significant effects were recorded, the comparison of means was performed with Duncan's multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

In Romania, the Portulaca first descriptions belong to Zach C. Panțu, *Plantele cunoscute de poporul român* (1906), like a "plant with a glabrous, branched stem, lying on the ground; fleshy leaves, yellowish flowers; fruits are globose capsules with reniform seeds".

According to Thalassinos and al. (2023) *P. oleracea* is a species well known for its ability to tolerate abiotic stresses and grows in soil burdened with anthropogenic activities. The wild variety has a taproot capable of penetrating soil layers to a depth of 25-30 cm and a

secondary root spreading at a diameter exceeding 30 cm. The colour of the stem varies from shades of green to reddish, it is glossy and juicy with a sour and salty taste. Characteristic of wild *P. oleracea* is the ascending circular branching of the main and second shoots around the stem, on a radius that can vary from 5 to 50 cm. Leaves are sessile, conical towards the base and rounded towards the tip; intense yellow small flowers with 3-7 mm long sepals and 3-8 mm long petals, united at the base. The inflorescence consists of 2-7 flowers that gradually mature (green capsules at immaturity and brownish at full maturity) (Figure 4).



a) b)

Figure 4. *Portulaca oleracea*: a) sprout detail; b) inflorescence detail

Purslane has been identified to contain the highest amount of omega-3 fatty acid of any green leafy vegetable and no cholesterol (Nemzer et al., 2020). Cultivated *P. oleracea* can provide more information for farmers as an alternative food source with nutritional benefits. Unlike the wild version, the cultivated form is vigorous with an erect port (Figure 5).



a) b)

Figure 5. *P. oleracea*: a) cultivated species with a strong, erect port; b) basal plant detail

It also has a strongly developed root in both horizontal and vertical planes, a tall habit, with 7-15 strongly branched main shoots.

During the study, we did not detect any diseases and/or pests that would cause significant damage to the crop. Through the breeding methods used, three genotypes were obtained: G1, G2 and G3.

All three genotypes presented a rich, branched and developed root in the horizontal plane, with a diameter between 25-30 cm and in the vertical plane with depths that varied between 15 and 23 cm (Figure 6).



Figure 6. *P. oleracea* root, cultivated species

The differences were observed in terms of biometric characteristics of aerial parts. The cultivars show a valuable characteristic, namely branching, which materializes in the formation of multiple stems with many inflorescences, which thus leads to increased plant mass. The bush has a lignified stem which gives it resistance and which varied between 1.7 to 3.1 cm in G1, from 1.8 to 3.3 cm in G2 (Figure 7) and between 1.7 to 2.5 cm in G3.



Figure 7. *P. oleracea* lignified stem at G2 genotype

Characteristic for cultivated genotypes are the erect, very vigorous and tall shoots. They have anthocyanin coloration in the lower half, are tender, crunchy, with a salty and sour taste. Easy harvesting is allowed because of the stem texture with a superior quality production (Figure 8). When the shoots are broken, a penetrating aroma specific to beetroot (*Beta vulgaris* var. *rubra*) or Malabar spinach (*Basella rubra*), known for their very high anthocyanin content, is released.



a) b)

Figure 8. *P. oleracea:* a) anthocyanin coloration shoots; b) strong yellow green colour leaves with spatula-shaped

The foliage is dense and the leaves are grouped in clusters of five at the top of every shoot. The leaves are succulent, fleshy, hairless and spatula-shaped. On the underside of the leaf, veins are weakly pronounced and the RHS colour chart (sixth edition) is 143A (strong yellow green) (Figure 8).

The length of leaves varies between 3.8-4.8 cm, in G1, 3.7-4.5 cm in G2 and 3.0-4.1 cm range in G3; the width varied between 1.1-1.7 cm, in G1, 1.2-1.6 cm range in G2 and 1.1.-1.6 cm in G3; the leaf weight varied between 0.25-0.44 g in G1, 0.22-0.34 g in G2 and between 0.30-0.38 g in G3; the leaf thickness was 0.1 mm for all genotypes;

The mean height plant varied between 60 to 80.4 cm in G1, from 64 to 86.4 cm in G2 and between 63 and 73 cm in G3. The canopy diameter varied between 44.0-61.5 cm in G1, from 52 to 67 cm in G2 and the range was 41.3 to 64 cm for G3. The inflorescences are grouped at the bottom of every shoot and are composed of 4-11 flowers that bloom in staggered order. The flowering runs from May to September. After flowering, they form capsules with gradual seed maturation (Figure 9).



Figure 9. *P. oleracea* inflorescences with flowers and seed capsules in different stages of maturation

The capsules are initially light green and as they mature they dehydrate and turn brown. The fruit is a dehiscent capsule. At full maturity, the dehiscent capsules suddenly open and the seeds inside are dispersed into the environment, usually at noon, when the thermal oscillations are maximum (Figure 10).



Figure 10. *P. oleracea* mature dehiscent capsule

On average, a main shoot weighs 126.43 g, has 9 inflorescences with 6.4 capsules each, a plant contains an average of 1239 capsules, each capsule containing 88.3 seeds, which means an average of 109492 seeds/ plant, due to the presence of very vigorous shoots with a rich foliage and a large number of inflorescences (7-60). The plant has a very high yield potential if harvested in a staggered way, breaking off the main shoots to stimulate the emergence of young ones. The average weight of a G2 mature plant is 1570 g. The seeds are staggered released on the soil in one season that can provide the seed requirement for the establishment of 2 ha of crop.

Crop technology

The genotypes obtained have a large, vigorous habit and require a larger space for nutrition and development, which is why the planting scheme was used at 70 cm between rows and 35-40 cm between plants per row (Figure 11).

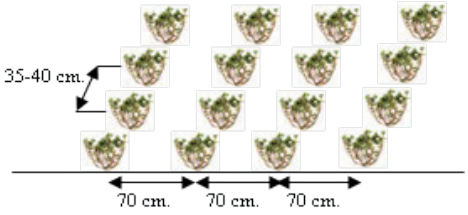


Figure 11. Cultivated *P. oleracea* - scheme culture: 70 cm between rows and 35-40 cm between plants/row

The sowing depth should be less than 0.5 cm, the seeds can germinate even uncovered at the soil surface (Vînătoru et al., 2019). Due to the very small size of the *Portulaca* seeds, several seeds are usually used for sowing, leading to a group of seedlings to be sown, which necessitates thinning as a mandatory operation after sowing. The plant's water needs are provided by the ramifications of the root system in horizontal plane, conferring resistance to drought. *P. oleracea* is characterized by tolerance and a very high potential of adaptability to thermohydric stress. The harvesting can start from May to late September, as the plant has a high regeneration power and thus ensures staggered production.

Main shoots chlorophyll determinations showed values ranging from 5.7 to 11.3 CCI.

Biometric observations at full maturity phenophase revealed maximum average values such as: canopy diameter 82.48 cm in G2, plant height 68.36 cm in G2, height sprout 2.46 cm in G2, diameter sprout 1.76 cm in G2.

Regarding the leaves dimensions, the measurements indicated average values such as: maximum leaf weight 0.34 g in G1, leaf length 4.24 cm and leaf width 1.38 cm in G3 (Table 1). Different small-case letters indicate significant differences among average values of biometric observations.

Table 1. Biometric measurements at cultivated *P. oleracea* growth aerial parts

Genotype	Canopy diameter (cm) CD	Plant height (cm) PH	Height sprout (cm) HS	Diameter sprout (cm) DS	Leaf weight (g) LW	Leaf length (cm) Ll	Leaf width (cm) LWdth
G1	68.28±6.30^{a}	57.3±9.36^{ab}	2.12±0.34^{a}	1.24±0.16^{a}	0.34±0.71^{a}	4.24±0.6^{b}	1.36±0.26^{a}
G2	82.48±4.11^{b}	68.36±4.61^{b}	2.46±0.66^{a}	1.76±0.33^{b}	0.28±0.50^{a}	4.08±0.35^{b}	1.36±0.18^{a}
G3	67±3.80^{a}	51±9.73^{a}	1.81±0.26^{a}	1.32±0.32^{a}	0.31±0.48^{a}	3.52±0.41^{a}	1.38±0.19^{a}

*Letters represent Duncan test results with 95% confidence interval and $p < 0.05\%$.

Regarding the development of aerial parts, the biometric determinations highlighted significant differences in the G2 genotype compared to the other two genotypes. Higher average values were recorded in G2 with a number of main sprouts of 8.4, a maximum level of sprout weight of 114.95 g and the highest number of inflorescences: 28.6 per sprout, and 192 per plant. G1 contains the maximum level of capsules/inflorescence: 8. G2 has the maximum number of seeds/capsule: 95 and of seeds/plant: 116998.2 (Table 2).

Table 2. Biometric measurements at cultivated *P. oleracea* development aerial parts

Genotype	Number of main sprouts NMS	Sprout weight (g) SW	Number of inflorescences/ sprout NIS	Number of inflorescences/ plant NIP	Number of capsules/ inflorescence NCI	Number of seeds/ capsule NSC	Number of seeds/plant NSP
G1	6.8±0.83^{a}	94.97 ± 4.76^{a}	17.6 ± 2.3^{a}	98.4 ± 20.35^{a}	8 ± 2.23^{a}	84.4 ± 14.74^{a}	71127 ± 35251.26^{a}
G2	8.4±1.14^{b}	114.95 ± 22.29^{b}	28.6 ± 5.85^{b}	192 ± 64.73^{b}	7.2±2.58^{a}	95± 18.39^{a}	116998.2± 16525.54^{b}
G3	5.7±1^{a}	94 ± 9.39^{a}	18.2 ± 2.38^{a}	118.8 ± 41.31^{a}	7.6±1.14^{a}	93.8 ± 12.02^{a}	82653± 25993.38^{a}

*Letters represent Duncan test results with 95% confidence interval and $p < 0.05\%$.

CONCLUSIONS

P. oleracea was submitted to breeding work at PGRB Buzau obtaining a valuable G2 variety with distinct phenotypic expressivity and high yield potential.

The cultivar can be successfully cultivated in an ecological system, being appreciated as food alternative, and also as a medicinal plant due to the active principles in its biochemical composition.

The culture technology for this species was developed.

In the future, PGRB Buzau will provide growers with both seeds and seedlings for testing.

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INFLUENCE OF CULTURE MEDIUM ON *IN VITRO* MICRO TUBER PRODUCTION IN *SOLANUM TUBEROSUM*

Ioana-Cătălina NICOLAE[1, 2], Oana VENAT[2], Adrian PETICILĂ[1], Vlad Mihai STANCU[3], Dorel HOZA[1]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[3]Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: catalina.nicolae@qlab.usamv.ro

Abstract

Potato (Solanum tuberosum) is highly susceptible to viral infections, which severely impact crop yields worldwide. In vitro microtuber production offers a valuable approach for producing disease-free planting material and conserving germplasm. This study evaluated the influence of culture medium composition - specifically varying sucrose concentrations and the presence of the cytokinin 6-Benzylaminopurine (BAP) - on microtuber induction in two blue-fleshed potato cultivars, 'Blue Congo' and 'Salad Blue'. Six medium variants were tested, combining three sucrose levels (3%, 4%, and 8%) with or without 0.5 mg/L BAP. Results showed that higher sucrose concentrations (8%) significantly enhanced microtuber formation frequency and number per explant, while BAP did not increase microtuber quantity but promoted larger and more elongated tubers. Both cultivars responded similarly across treatments, with media containing 8% sucrose and BAP yielding the most effective microtuber production within two months. These findings underscore the critical role of culture medium composition in optimizing in vitro microtuberization for efficient propagation and preservation of potato germplasm.

Key words*: germplasm conservation, growth regulators, micropropagation, potato, slow growth storage.*

INTRODUCTION

Potato (*Solanum tuberosum*) is a species extremely vulnerable to various infections, especially to viral ones. The increasing prevalence of insect vectors – as well as the limited effectiveness or absence of reliable seed-testing systems – greatly amplifies the impact of viral diseases in potato production. These viral infections can lead to yield reductions exceeding 50% of the crop's potential production (Harahagazwe et al., 2018; Kreuze et al., 2020). Although more than 50 different viruses are known to infect potato worldwide, only a small number of them are responsible for the major yield losses reported globally (Kreuze et al., 2020). Potato Virus Y (PVY) is the most damaging potato virus, causing up to 80% yield loss and it is transmitted by more than 50 aphid species, specially *Myzus persicae*, the highly efficient and globally widespread main vector (Robert & Bourdin, 2001; Kerlan, 2006; Quenouille et al., 2013). PVY also infects many other cultivated solanaceous crops and numerous weed species, broadening its impact on agriculture and horticulture (Kaliciak & Syller, 2009). Another major pathogen, Potato Leaf Roll Virus (PLVR), can reduce yields by up to 90% and is likewise spread primarily by *Myzus persicae*, though in a slower manner, due to its circulative transmission (Quenouille et al., 2013; Ștef et al., 2024). With the aphid populations expected to expand globally, the risk of infections with PVY, PLVR, and other aphid-borne viruses is projected to rise (Norse & Gommes, 2003; Jones, 2009; Cowan et al., 2023).

In potato seed production, micropropagation plays a vital role in maintaining genetic fidelity and ensuring the material is disease-free, especially regarding viral diseases. This process typically involves three main steps: (1). Generation of virus free stock material: achieved through virus elimination techniques, such as

meristem culture, thermotherapy, cryotherapy; (2). Multiplication through micropropagation techniques: once a stock of pathogen-free plantlets is established, they are multiplied under sterile laboratory conditions, using different types of culture media and growth regulators and/or obtaining microtubers on media with high concentrations of sucrose; (3). Cultivating the resulting microtubers as nuclear stock, in greenhouses, to obtain disease-free potato seed tubers (Naik & Karihaloo, 2007).

The Murashige & Skoog (1962) is regarded as the most widely used medium for potato micropropagation, and it is frequently applied without the inclusion of plant growth regulators (Naik & Karihaloo, 2007). While the standard MS vitamin mixture is typically retained, adjustments may be made – such as increasing the thiamine concentration or incorporating additional vitamins, such as biotin (0.5 mg/L) – to further improve growth performance (Abdullateef et al., 2009). In most cases, culture medium is solidified using agar or gelrite; however several studies have reported improved growth performance when liquid media are used instead (Del Avila et al., 1996; Jiménez et al., 1999; Abdullateef et al., 2009). A common approach in potato micropropagation involves first establishing a sufficient number of cultures on a semi-solid medium, followed by transferring them to a liquid medium for large-scale multiplication (Naik & Karihaloo, 2007; Abdullateef et al., 2009) found that nodal segments of various potato cultivars ('Ackersegen', 'Blaue Schweden', and 'Frühnudel') produced more and taller shoots, with a greater number of axillary buds when grown in liquid media with very low BAP concentrations in temporary immersion systems, compared with those maintained on semi-solid media. These improvements are likely due to more efficient nutrient absorption and enhanced gas exchange, which reduces ethylene buildup within the culture vessels (Etienne & Berthouly, 2002; McAlister et al., 2005). Potato is a species known for its sensitivity to ethylene when cultivated in in vitro conditions, especially in cuture vessels that do not allow gas exchange (Hussey & Stacey, 1981; Zobayed, 2001; Jackson et al., 1991; Sarkar et al., 1999). Growth regulators may be included in the medium for multiplication, though typically at low concentrations (Abdullateef et al., 2009) The crucial role of elevated sucrose levels in promoting *in vitro* microtuber formation is widely documented by several authors (Mohamed Ali & Esmail, 2011; Cioloca et al., 2023; Develi & Miler, 2023). Sucrose levels used for microtuber induction typically range from 3% to 8% (Yu et al., 2000; Dhital & Lim, 2012; Develi & Miler, 2023), and concentrations as high as 9% have also proven effective in this process (Gudeva et al., 2016) Cytokinins – including 6-Benzylaminopurine (BAP) and kinetin (KIN) – also play a major part in this process, as reported by numerous studies (Pelacho & Mingo-Castel, 1991; Dhital & Lim, 2012; Abu Zeid et al., 2022). In *Solanum* species, kinetin had been shown to promote microtuber formation and to act synergistically with other cytokinins such as BAP (Anjum & Villiers, 1997).

For the *in vitro* microtuberization experiments presented in this paper, the cultivars 'Blue Congo' and 'Salad Blue', both characterized by their blue-fleshed tubers, were used. Blue pigmented potato cultivars are relatively uncommon and regarded more as a gourmet speciality, enjoying notable popularity in high-end restaurants. Nonetheless, farmers are often reluctant to cultivate them due to their considerably lower yields, compared to white- and red-fleshed cultivars, as well as their high susceptibility to diseases during tuber propagation (Witbooi et al., 2021; Bvenura et al., 2022). The intense blue pigmentation of this varieties results from the presence of anthocyanins – malvidin and petunidin, along with delphinidin, peonidin, pelargonidin, and cyanidin (Han et al., 2006; Kita et al., 2013). Moreover, studies on blue-fleshed potato cultivars have shown that they contain higher levels of phenolic acids, flavonoids, and coumarins, compared to white - and yellow - fleshed cultivars (Murniece et al., 2013; Ru et al., 2019; Saar-Reismaa et al., 2020).

'Blue Congo', also referred to by various names including 'Blauer Schwede', 'Blaue Schweden', 'Idaho Blue', 'Purple Congo', and 'Blå Kongo', is a moderately late-maturing heritage potato variety un uncertain origin, believed to have emerged either in Europe or in the Americas. Yield data indicate that this cultivar demonstrates stable performance under a wide

range of environmental conditions (www.spk.fi; www.heritagepotato.ca). Tubers typically have a round-oval to long-oval shape. This cultivar is known to be susceptible to *Streptomyces scabies* (Dees et al., 2013) and has a moderate resistance to *Phytophthora infestans* (Pazderů & Hamouz, 2017). 'Salad Blue' is a potato cultivar that is distinguished by its high resistance to the golden nematode *Globodera rostochiensis* (The European Cultivated Potato Database - www.europotato.org/) and for its striking blue skin and flesh. Originating from Scotland, it is an early-maturing and hardy variety, known for its drought tolerance and ability to maintain good yields under adverse environmental conditions. The tubers are typically oval in shape, featuring blue to purple skin and purple flesh accented with white streaks (Pęksa et al., 2013; Witbooi et al., 2020; Nagy et al., 2023).

MATERIALS AND METHODS

Plant material consisted of *in vitro* cultivated shoots of 'Salad Blue' (Figure 1) and 'Blue Congo' varieties. The shoots were subcultured every 3-4 months on MS medium (Murashige & Skoog, 1962) with an increased concentration in vitamins, as used in the formulation described by Gamborg et al. (1968).

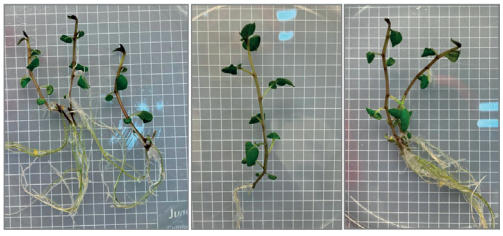


Figure 1. *In vitro*-obtained shoots of 'Salad Blue' cultivar used for *in vitro* tuberization

For the *in vitro* production of microtubers, six culture media variants were prepared, each containing different combinations of sucrose and 6-Benzylaminopurine (BAP), as detailed in the table below (Table 1). The mineral composition followed the formulation of (Murashige & Skoog, 1962), with a modified concentration of thiamine HCl (1 mg/L). All stock solutions used in the media for this experiment were prepared separately for each reagent. Macroelements were formulated at ten times their final working concentration, while microelements were prepared at 1,000-fold strength, with the exception of the sodium molybdate dihydrate ($NaMoO4 \cdot 2H_2O$), prepared at 1,690 mg/L, and FeNaEDTA, prepared at 7,340 mg/L.

Table 1. Composition of the culture media used for obtaining *in vitro* microtubers

No.	Composition of culture medium	Culture medium variants					
		T1	T2	T3	T4	T5	T6
1.	6-Benzylaminopurine (BAP) (mg/L)	0	0.5	0	0.5	0	0.5
2.	Sucrose (g/L)	30	30	40	40	80	80
3.	Agar (g/L)	7	7	7	7	7	7

Vitamins and 6-Benzylaminopurine (BAP) were prepared as stock solutions at 100 mg/L. The medium was dispensed into 750-ml glass jars, with approximately 100 ml in each jar, and then covered with lids with filters, to allow air exchange and prevent contamination. BAP was sterilized together with the rest of the components, in the autoclave, for 121°C for 20 minutes.

RESULTS AND DISCUSSIONS

Percentage of explants producing microtubers

The most effective media for inducing microtuber formation were those containing higher sucrose concentrations and supplemented with cytokinin, specifically media variants T5 and T6, which achieved 100% micro tuber formation across all shoots in both 'Salad Blue' and 'Blue Congo' cultivars (Figure 2). On these media variants, tubers developed within two months of culture (Figure 2). Media containing 3% sucrose not only exhibited the lowest tuberization rates, but also required a significantly longer initiation period; in the case of variant T1, microtuber formation occurred only after 4 months.

Similar findings have been reported by other researchers, highlighting that the carbon source and growth regulators are the primarily factors influencing *in vitro* potato tuberization (Momena et al., 2014). Studies by Gudeva et al. (2016) have shown that increasing the concentration of sucrose up to 90 g/L strongly enhanced microtuber formation.

However, plant growth regulators also played a crucial role, as the addition of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) to a medium

containing only 60 g/L sucrose resulted in over 85% microtuberization in the case of 'AgriaSR' cultivar. Hoque, (2010) reported strong tuberization responses in segments grown with 6% sucrose and kinetin. Conversely, Gautam et al. (2021) found better results on hormone-free medium - producing more microtubers per explant, with greater average weight and diameter - compared to a medium supplemented with 10 mg/L BAP. Microtuber formation can occur at lower sucrose concentrations (30-40 g/L), but the frequency and tuber size are notably reduced.

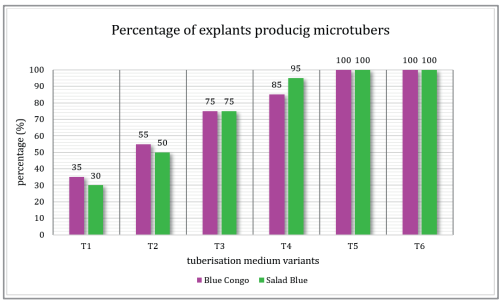


Figure 2. Percentage of explants that produced microtubers across the six different micro tuberization media formulations

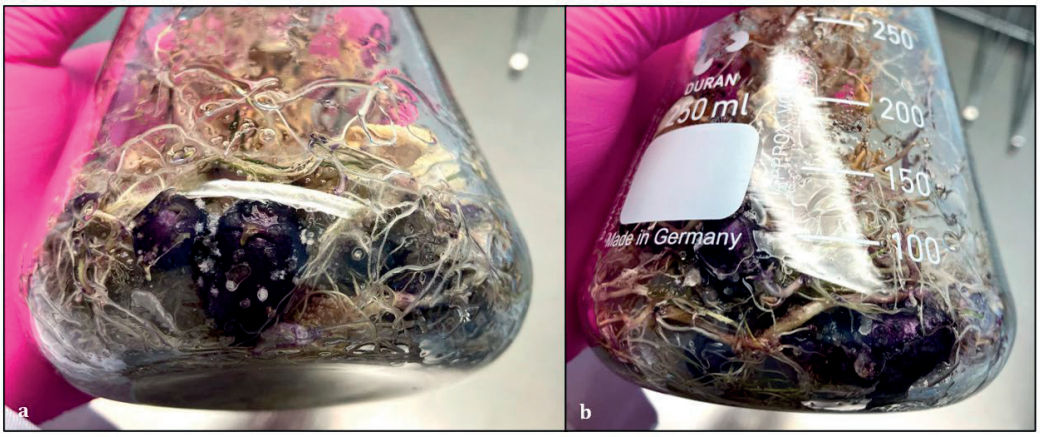


Figure 3. a,b. Microtubers obtained on tuberization media with 80 g/L sucrose and 6-Benzylaminopurine (BAP)

Dessoky et al. (2016) achieved over 50% tuberisation and an average of 2.9 microtubers per shoot using standard sucrose levels (3%), combined with 7 mg/L BAP and 0.2 mg/L KIN. Borna et al. (2019) succesfully induced microtubers in the 'Diamant' variety by culturing microcuttings on media with high plant growth regulator levels and normal sucrose. Conversely, Abu Zeid et al. (2022) found that excessively high sucrose concentrations (120-160 g/L) negatively affect *in vitro* tuber production.

In our study, the percentage of tuberization was comparable between the two cultivars, showing no statistically significant differences.

Average microtuber count per explant

The number of microtubers produced per explant was strongly affected by sucrose concentration, while the influence of BAP was comparatively minor. The highest results were achieved on media with elevated sucrose levels - specifically variants T5 and T6 – which produced averages pf 4.13 and 4.08 microtubers/explant, with no statistically significant differences between them. Microtuber production did not vary notably between cultivars; both 'Blue Congo' and 'Salad Blue' exhibited similar responses. The mean value of 4.25 microtubers/explant, was recorded for 'Salad Blue' in the cytokinin-supplemented medium containing 8% sucrose, followed closely by 'Blue Congo' cultured on a hormone-free medium with the same sucrose concentration, averaging 4.20 microtubers/explant (Figure 4).

In contrast, the lowest averages were observed in 'Blue Congo', with 1.60 microtubers/explant on variant T1 (standard sucrose, no growth regulators) and 1.70 on variant T2 (standard

sucrose with BAP). Media containing 40 g/L sucrose resulted in slightly better outcomes – 1.88 microtubers/ explant on T3 and 2.15 in T4 – roughly half the number obtained on the media with 80 g/L sucrose

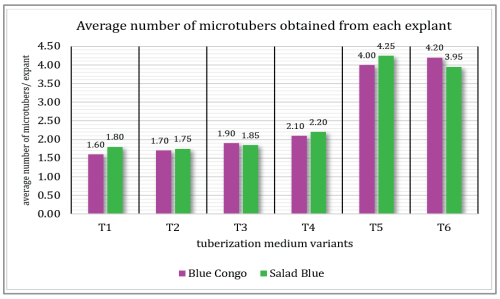


Figure 4. Average number of microtubers obtained per explant

Comparable findings have been reported by Develi & Miler (2023), who achieved between 1.0 and 4.25 microtubers per explant in 'Aruba' and 'Oberon' cultivars, using only media with 4% sucrose and no growth regulators, under varied lighting conditions. Simillarily, (Mohamed Ali & Esmail, 2011) observed that single-node explants from several potato cultivars produced between 0.48 and 0.70 microtubers/ explant on medium containing 6% under different illumination sources.

Overall, BAP did not show a significant influence on thee quantitative aspect of microtuber production. Gudeva et al. (2016) reported averages ranging from 0.61 to 3.2 microtubers/ explant on media with 3-9% sucrose, combined with elevated levels of BAP and NAA. Simillarily, Develi & Miler (2023) achieved up to 4.25 microtubers per explant in 'Aruba' cultivar grown on medium supplemented only with 4% sucrose.

Average microtuber growth

Although BAP appeared to have a minimal impact on the number of microtubers produced from each explant, subsequent parameters revealed notable differences among the media variants, depending on the presence or absence of BAP. Scheirer-Ray-Hare (SRH) test indicated significant variation in both microtuber length p = 5.84124E-30) and width (p = 1.59171E-29). No considerable differences were observed between the two studied cultivars. Regarding the microtuber length, the highest values were obtained on variant T6, averaging 16.97 mm, followed by T5, with an average of 13 mm. Dunn's tst indicate a significant difference between these two variants, underscoring the role of cytokinins in microtuber development (Figure 6.a). The smallest tubers appeared under conditions of low sucrose and in the absence of plant growth regulators, with the lowest means recorded in T1 (7.13 mm) and T3 (9.67 mm). Intermediate lenghts were seen in the shoots cultivated with lower sucrose but supplemented with BAP: 10.18 mm for T2 and 11.56 for T4, with no statistically significant differences between these two variants.

Microtubers exhibited a narrower width, indicating they tend to be more elongated than round or isodiametric in shape (Figure 5)



Figure 5. Microtubers obtained on different tuberization medium variants: a. T6; b. T4 and c. T1

The pattern for this parameter mirrored previous observations, with the greatest measurements recorded on variant T6, averaging 13.57 mm across both cultivars, and variant T5, averaging 11.79 mm, showing a significant difference between them.

Smaller widths were observed in variants T1 (6.47 mm) and T3 (8.00) (Figure 6.b).

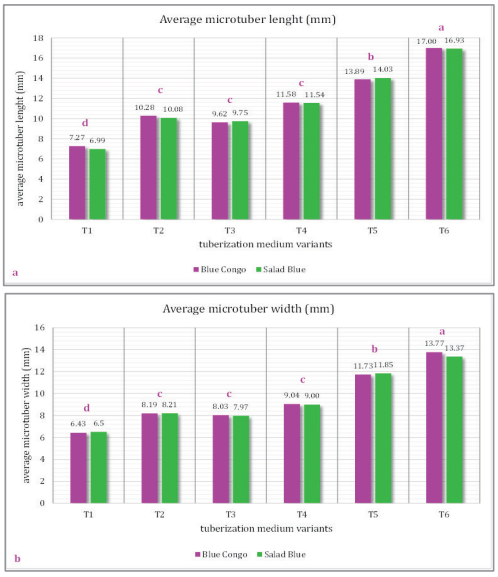


Figure 6. a. Average microtuber length (mm) on the six tuberization medium variants and b. Average microtuber width (mm) on the six tuberization medium variants

Findings indicate that both the levels of sucrose and the presence of cytokinin play crucial roles in microtuber development. Yu et al. (2000) reported a significant decline in microtuber growth rates when sucrose concentration was reduced from 8% to 4% in bioreactor cultures. Regarding the effect of plant growth regulators on tuber growth Gautam et al. (2021) found that explants grown without hormones but with 8% sucrose produced larger microtubers compared to those cultured on media with high BAP concentrations (10 mg/L).

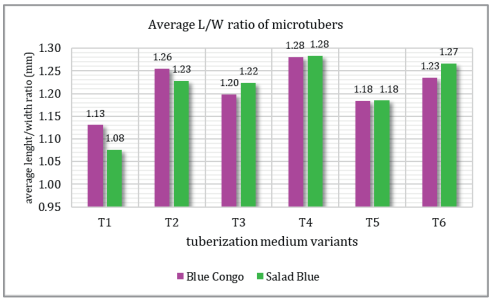


Figure 7. Average length/width ratio of microtubers obtained on the tuberisation media

Similarly, Gudeva et al. (2016) observed smaller microtubers – averaging 5 to 5.5 mm in length depending on the variety – when using media enriched with high levels of BAP and NAA, combined with 60-90 g/L sucrose. Interestingly, the shape of the microtubers was affected by the presence of growth regulators. Analysis pf the length-to-width ratio across all measured tubers revealed significant differences between various media formulations. Microtubers grown on media containing BAP tended to be more elongated (Figure 7), with the greatest length-to-width ratio observed in the T4 medium variant. These findings indicate that both sucrose concertation and the presence of cytokinins play crucial roles in microtuber development.

CONCLUSIONS

Microtubers are favoured over tissue-cultured plantlets, because they are easier to plant, have lower mortality rates, and do not require an acclimatization phase. However, they present a drawback with their longer dormancy period compared to field-grown tubers leading to a delayed sprouting (Westra et al., 2020). On the other hand, this prolonged dormancy is beneficial for in vitro conservation (Estrada et al., 1986)

The study demonstrated that the composition of the culture medium significantly influences in vitro microtuber production in *Solanum tuberosum*. Higher sucrose concentrations (8%) notably enhanced both the frequency of microtuber formation and the average number of microtubers per explant, compared to lower sucrose levels (3% or 4%). The addition of the cytokinin 6-Benzylaminopurine (BAP) did not significantly increase the number of microtubers but positively affected microtuber size and shape, producing larger and more elongated tubers. Both Blue Congo and Salad Blue cultivars responded similarly to the tested media, indicating no major cultivar-specific differences in microtuber production. Overall, media with high sucrose combined with BAP (T5 and T6 variants) were the most effective for rapid and abundant microtuber induction, supporting their use for disease-free potato propagation and germplasm conservation

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A COMPREHENSIVE REVIEW OF MINT ESSENTIAL OILS – COMPOSITION, QUALITY AND APPLICATIONS

Sabina PINTILIA[1, 2], Elena PETCU[2], Niculina IONESCU[2], Violeta Alexandra ION[3], Liliana BĂDULESCU[2, 3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Agricultural Research and Development Institute-Fundulea, 1 N. Titulescu str, Călărași, Romania
[3]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: liliana.badulescu@usamv.ro

Abstract

Mint essential oils, extracted from various species of Mentha, are highly valued for their aromatic, therapeutic, and industrial uses. This comprehensive review examines the chemical composition, quality assessment techniques, and broad applications of mint essential oils. Key bioactive compounds such as menthol, menthone, and isomenthone play a pivotal role in the characteristic aroma and functional properties of these oils. Emerging tools, including chemometric models combined with spectroscopy, promise rapid, non-destructive quality control. Advanced techniques like liquid chromatography-electrospray ionization time-of-flight mass spectrometry (HPLC-ESI-TOF/MS), headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS), and ATR-FTIR spectroscopy offer improved detection of adulteration and detailed profiling of both volatile and non-volatile components. Nonetheless, factors such as processing, storage, and environmental conditions can significantly alter oil composition, emphasizing the need for standardized protocols to ensure consistent quality. These advancements are set to solidify the role of mint essential oils in aromatherapy, pharmaceuticals, and diverse industrial applications.

Key words*: esential oil, Mentha sp., analytical techniques, volatile and non-volatile compounds, quality.*

INTRODUCTION

Peppermint (*Mentha* × *piperita*), a valued member of the mint family (Lamiaceae), is widely recognized for its medicinal, culinary, and industrial significance. Renowned for its distinct aroma and therapeutic properties, it has been used for centuries in traditional medicine and modern applications (Liang et al., 2024). This versatile plant is a cornerstone of the global essential oils (EOs) industry, valued at over $400 million annually. Its adaptability to diverse agro-climatic conditions and extensive use across multiple sectors underscore its economic and pharmacological importance (Sercan et al., 2025).

Taxonomically, peppermint (*Mentha* × *piperita*) belongs to the Mentha genus, which includes approximately 42 species (Mokhtarikhah et al., 2020), with the most common species illustrated in Figure 1. It is a natural hybrid of *Mentha spicata* (spearmint) and *Mentha aquatica* (watermint) and is cultivated worldwide for its essential oil.

Peppermint essential oil (EO) is a complex mixture of volatile compounds, primarily terpenes and phenols, extracted from various plant parts through methods like steam distillation. Its key bioactive components, including menthol, menthone, and carvone, contribute to its distinct properties, with composition and quality influenced by geographical and climatic factors (Mahendran et al., 2021). The oil is valued not only for its characteristic fragrance but also for its potent bioactive effects.

Recognized for its antimicrobial, antioxidant, and therapeutic properties, peppermint EO is widely used in food preservation, pharmaceuticals, and cosmetics. Unlike synthetic alternatives, it is biodegradable,

environmentally friendly, and classified as generally recognized as safe (GRAS), further increasing its industrial appeal (Burt, 2004).

Beyond its medicinal and aromatic properties, peppermint has been traditionally used to treat gastrointestinal and respiratory ailments and as a carminative and sedative agent. Today, it is extensively incorporated as a flavoring agent in food and beverages, as well as in cosmetics and oral hygiene products (Saleem et al., 2018). The rising demand for natural, eco-friendly solutions has amplified interest in its pharmacological potential and industrial applications. These factors make peppermint EO a subject of extensive scientific research, particularly in the fields of pharmacology and quality control.

Figure 2 illustrates the prominence of the Lamiaceae family in essential oil production, alongside contributions from families such as Apiaceae and Lauraceae. Additionally, smaller families like Zingiberaceae and Rutaceae contribute to the diverse sources of essential oils used in pharmacology and traditional medicine.

This review provides a comprehensive analysis of mint essential oils, emphasizing advanced analytical techniques, quality control measures, and methods for detecting adulteration. It also compares traditional and modern extraction techniques, evaluating their impact on oil composition and bioactivity. Beyond pharmaceuticals and cosmetics, peppermint essential oil is explored for its industrial and environmental applications, with a key focus on linking specific bioactive compounds to pharmacological properties. Encapsulation techniques are also discussed for improving the stability and efficacy of these bioactive components.

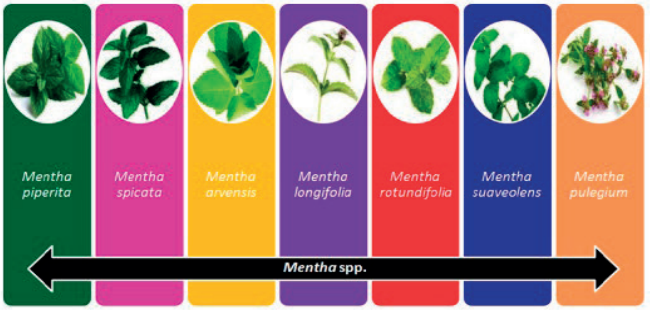


Figure 1. Common species of *Mentha* (Source: Eftekhari et al., 2021)

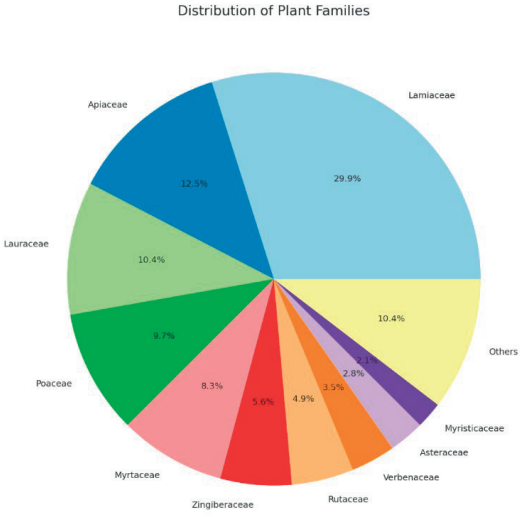


Figure 2. Distribution of various plant families used for essential oils (Source: Liang et al., 2024)

EOs EXTRACTION METHODS

Essential oils from herbs and spices possess strong antioxidant and antimicrobial properties, but their quality depends largely on the extraction method. Traditional techniques like steam distillation and hydrodistillation are widely used due to their simplicity and cost-effectiveness; however, they often require high temperatures and large amounts of solvents, which can degrade heat-sensitive compounds (Thuyen et al., 2023; Abbas et al., 2024).

In contrast, modern methods such as supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE) offer higher efficiency and better preservation of bioactive compounds but require expensive equipment and precise optimization to prevent degradation (Rasekh et al., 2023; Pogorzelska-Nowicka et al., 2024). Emerging techniques like ultrasound-assisted extraction (UAE) strike a balance between efficiency and sustainability by improving yield while reducing energy consumption. However, improper optimization can still lead to the breakdown of sensitive compounds (Pogorzelska-Nowicka et al., 2024). The selection of an extraction method depends on factors such as the oil's chemical composition, intended application, and economic feasibility. Table 1 compares various extraction techniques, outlining their principles, advantages, disadvantages, and examples of commonly extracted oils.

Table 1. Comparison of Different Extraction Methods for Essential Oils

Extraction Method	Principle	Advantages	Disadvantages	References
Steam Distillation	Steam passes through plant material, vaporizing the volatile oils, which are then condensed.	Simple, widely used, and cost-effective.	Can cause thermal degradation of sensitive compounds.	Thuyen et al., 2023
Solvent Extraction	Solvents like ethanol or hexane are used to dissolve oils, followed by solvent removal.	Can extract oils from delicate flowers and non-aromatic plants.	Residual solvent can be harmful; more complex process.	Thuyen et al., 2023
Supercritical Fluid Extraction (SFE)	Uses supercritical CO_2 to extract oils.	High efficiency, preserves sensitive compounds, no solvent residue.	Expensive equipment, high energy requirements.	Rasekh et al., 2023
Hydrodistillation	Similar to steam distillation but with the plant material submerged in water.	Effective for large quantities, relatively simple.	Loss of some volatile compounds in water, less suitable for delicate oils.	Abbas et al., 2024
Microwave-Assisted Extraction (MAE)	Uses microwave radiation to assist in extracting oils.	Fast extraction, less solvent required.	May cause uneven heating, leading to degradation of some compounds.	Pogorzelska-Nowicka et al., 2024
Ultrasound-Assisted Extraction (UAE)	Uses ultrasonic waves to break cell walls and release oils.	Fast, energy-efficient, can improve yield.	May cause degradation of sensitive compounds if not optimized.	Pogorzelska-Nowicka et al., 2024

EOs QUALITY EVALUATION

The quality assessment of EOs is critical due to their high volatility and susceptibility to oxidation. Various techniques have been developed to evaluate EO quality and detect adulteration. ***Sensory evaluation*** involves both human sensory analysis and electronic devices, such as electronic noses and tongues, to assess aroma and appearance. ***Physico-chemical analysis*** measures intrinsic properties like density, optical rotation, and refractive index to ensure quality standards (Ayu et al., 2024 a,b). Chemical composition analysis employs chromatography, mass spectrometry, and spectroscopy to identify and quantify volatile and non-volatile components. Advanced methods, including Raman spectroscopy, Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS), and Nuclear Magnetic Resonance (NMR), provide precise and rapid analysis (Sousa et al., 2022). Combination approaches integrate multiple techniques for comprehensive and accurate quality control, ensuring consumer safety and market stability (Gabetti et al., 2021).

Sensory evaluation of essential oils

Sensory evaluation of EOs involves human sensory analysis and electronic sensor technologies to assess aroma and appearance. It includes biological sensory evaluation, conducted by trained evaluators, and electronic chemical sensing, using devices like electronic noses and tongues (Albuquerque et al., 2016). Both methods play a crucial role in determining EOs quality and authenticity. While human sensory analysis is reliable, it requires careful selection and training of evaluators. Combining human expertise with electronic devices provides a more comprehensive and objective assessment (Gabetti et al., 2021).

Electronic noses and tongues have gained traction in the food industry due to their speed and efficiency compared to human evaluation (Albuquerque et al., 2016). These devices utilize chemical sensors and pattern recognition systems to analyze samples, generating fingerprint data for both qualitative and quantitative assessments. They are effective in establishing databases, detecting adulteration, and classifying EO quality (Rasekh et al., 2023). Electronic noses can differentiate essential oils, detect adulteration, and assess the quality of mint oil. These technologies offer a rapid, reliable, and objective approach to EO quality evaluation (Gabetti et al., 2021).

Physicochemical properties evaluation

To ensure accurate and comprehensive EO quality assessment, physicochemical properties are evaluated using standardized methods. These properties provide vital insights into the composition, purity, and potential adulteration of essential oils (Cárdenas et al., 2020).

Key physicochemical properties include:

- *Density*, which measures the mass per unit volume of an EO, helps assess its purity and composition.
- *Optical rotation*, which refers to the deviation of light as it passes through the EO, is essential for distinguishing synthetic oils from pure ones. Synthetic oils often exhibit different optical rotation values compared to authentic EOs.
- *Refractive index*, which indicates how light bends as it passes through the oil, offers a quick and reliable method for evaluating EO purity and detecting adulteration (Guil-Guerrero et al., 2017).

These physicochemical properties are essential for quality control and detecting adulteration. For example, the peppermint oil adulterated with turpentine will show a lower freezing point than the standard 10.5°C, indicating possible tampering (Cárdenas et al., 2020).

Chemical properties evaluation

For precise and objective evaluations of essential oils (EOs), advanced techniques like chromatography, mass spectrometry, and spectroscopy are crucial. These methods detect and identify volatile, chiral, and non-volatile compounds, key to understanding the true composition of EOs. Volatile compounds, responsible for the distinct aroma of EOs, are especially important for quality control (Łyczko et al., 2023). Techniques like liquid chromatography-electrospray ionization time-of-flight/mass spectrometry (HPLC-ESI-TOF/MS) (El Omari et al., 2024) and headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME/GC-MS) (Kataoka et al., 2000) provide

detailed analysis of these compounds, linking chemical composition with aroma profiles (de Sousa et al., 2023).

Chiral compounds, such as terpenes, significantly influence the aroma and flavor of EOs. Analyzing enantiomeric ratios using chiral gas chromatography can confirm authenticity, with deviations, such as in α-pinene, signaling potential adulteration (Gabetti et al., 2021).

Non-volatile compounds, though less relevant to aroma, offer vital insights into authenticity and overall quality. Sterols, fatty acids, and waxes, detectable via high-performance liquid chromatography (HPLC), serve as valuable markers for adulteration. This method is particularly effective in distinguishing authentic citrus oils, such as orange, mandarin, and lemon, from adulterated samples (Tranchida et al., 2013). The article written by Boren et al. (2015), highlights various methods used to detect adulteration and the challenges associated with ensuring the purity of essential oils. This essay examines the key aspects of adulteration in mint essential oils and the analytical techniques used to maintain their authenticity.

When comparing analytical methods for essential oils, Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) both focus on volatile compounds but differ in depth. GC-MS adds mass-to-charge ratio analysis, enhancing its ability to identify a broader range of compounds, such as menthol, menthone, and pulegone (Łyczko et al., 2023).

In the study by El Omari et al. (2024), these techniques were employed to characterize the chemical composition of *Mentha piperita* (peppermint) essential oils. The analysis identified menthol and menthone as predominant constituents, underscoring the efficacy of GC and GC-MS in profiling volatile substances in essential oils. Fourier Transform Infrared Spectroscopy (FTIR) offers a unique approach, identifying functional groups in molecules, making it useful for characterizing terpenes and alcohols (Kucharska-Ambrożej et al., 2021).

Headspace Solid-Phase Microextraction (HS-SPME) coupled with GC excels in extracting volatile compounds from a sample's headspace, ideal for analyzing compounds like menthol and carvone (Łyczko et al., 2023; Nasreddine et al., 2024). Meanwhile, Thin-Layer Chromatography (TLC), though less precise, offers a simpler, cost-effective method for separating compounds like menthol and terpenes (Radu et al., 2023).

For detecting metal content, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) effectively identifies heavy metals such as lead and mercury in EOs, ensuring safety and quality (Mei et al., 2024). In contrast, Refractive Index (RI) Measurement offers a quick and cost-effective assessment of EO purity and concentration, especially for oils like mint (Mahboub et al., 2020).

ATR-FTIR spectroscopy, combined with advanced chemometric models such as PCA-LDA, PLS-DA, and SIMCA, has proven effective for detecting adulterations in EOs, particularly Mentha arvensis oil. This method provides a rapid, non-destructive approach for detecting adulterants with high accuracy (Kucharska-Ambrożej et al., 2021). The combination of UV-VIS spectrophotometry and ATR-FTIR spectroscopy has further demonstrated its strength in mint quality control, achieving accuracy rates of 60-80% depending on data and harvest season (Kucharska-Ambrożej et al., 2019). The development of portable ATR-FTIR spectrophotometers may further enhance on-site EO analysis, making these methods more accessible for routine quality assurance (Kucharska-Ambrożej et al., 2021).

Essential oils (EOs) are analyzed using various techniques, each suited for specific compounds as described in Table 2. Gas Chromatography (GC) - FID separates volatile compounds like menthol and menthone based on their interaction with a column. GC-MS combines GC with mass spectrometry to identify compounds such as carvone and pulegone by their mass-to-charge ratio. FTIR detects functional groups in terpenes and alcohols by measuring infrared light absorption. Other methods like HS-SPME-GC, TLC, and Mass Spectrometry (MS) also contribute to comprehensive EO analysis, ensuring accurate composition and purity. These techniques support quality control and safety in EO applications.

Table 2. Analytical methods used for essential oils (EOs) mint analysis

Analytical Method	Principle	Typical Compounds Analyzed	References
Gas Chromatography (GC-FID)	Separation of volatile compounds based on their interaction with a column.	Monoterpenes (e.g., menthol, menthone)	Loi et al., 2024
Gas Chromatography-Mass Spectrometry (GC-MS)	GC coupled with mass spectrometry to identify compounds based on mass-to-charge ratio.	Menthol, menthone, carvone, pulegone	Mogoşan et al., 2017
Headspace Solid-Phase Microextraction (HS-SPME) Gas Chromatography (GC)	Extraction of volatile compounds from a sample's headspace followed by GC analysis.	Volatiles such as menthol, carvone	Łyczko et al., 2023; Nasreddine et al., 2024
Fourier Transform Infrared Spectroscopy (FTIR)	Measures absorption of infrared light to identify functional groups in molecules.	Functional groups in terpenes, alcohols	Kucharska-Ambrożej et al., 2021
Thin-Layer Chromatography (TLC)	Separation of compounds on a stationary phase based on their different affinities.	Menthol, menthone, carvone, terpenes	Radu et al., 2023
Mass Spectrometry (MS)	Ionizes compounds to measure their mass-to-charge ratio and deduce structure.	Terpenes, phenolic compounds	Rasekh et al., 2023
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	Measures metal content in a sample by ionizing the sample and analyzing mass-to-charge ratios.	Heavy metals (lead, mercury)	Mei et al., 2024
Refractive Index (RI) Measurement	Measures the refractive index of the sample to assess purity or concentration.	Concentration of essential oils (e.g., mint)	Mahboub et al., 2020

COMPOUNDS OF ESSENTIAL MINT OIL

EOs from spearmint (*Mentha spicata*) have been extensively studied for their chemical composition, ecological adaptations, and potential applications. High EO content is observed in landraces grown at lower altitudes and in arid climates, where rainfall is minimal, reflecting specific ecological adaptations (Sangwan et al., 2001). Gas chromatography-mass spectrometry (GC-MS) has identified 40 components in spearmint EO, with oxygenated monoterpenes comprising 12.6% to 86.61% of the total composition. The major constituents are carvone (0.1% to 73.4%) and menthone (0.02% to 62%), consistent with findings by Kizil et al. (2010) and other studies (Zheljazkov et al., 2010).

Cluster analysis of 25 landraces revealed three distinct clusters based on EO composition and ecological traits, illustrating the diversity within the species. The biosynthesis of these EOs involves terpene synthase and cyclase enzymes, which produce terpenes and phenylpropanoids through the pyruvate-mevalonate and shikimate pathways (Kizil et al., 2010). Variations in carvone and limonene content across global regions further emphasize the role of environmental factors in EO composition (Snoussi et al., 2015).

The chemical composition of mint EO is complex, with oxygenated monoterpenes being the most abundant class. Carvone, a major compound, contributes significantly to both the aroma and flavor of mint EO, ranging from 0.1% to 73.4% (Hussain et al., 2010). Menthone, another prominent compound, is found in concentrations of 0.02% to 62%, varying across landraces (Eftekhari et al., 2021). Other oxygenated monoterpenes, such as neo-dihydrocarveol (0.08-54.7%) and pulegone (0.2-32.2%), show considerable variation based on ecological factors (Shrigod et al., 2016).

In addition to carvone and menthone, compounds like 1,8-Cineole (eucalyptol) (0.1-46.28%) and iso-menthone (0.03-39.1%) are present in varying concentrations, depending on regional varieties (Padalia et al., 2013; Arzani et al., 2005). Menthol (up to 3.02%) is found in specific samples, contributing to the cooling sensation associated with mint (Dambrauskienė et al., 2008). Research on *Mentha rotundifolia* essential oils revealed the presence of two chemotypes: one characterized by high

piperitone oxide content and the other by elevated piperitenone levels (Brada et al., 2007). Monoterpene hydrocarbons, including limonene (1.9-31%), show a negative correlation with carvone, resulting in distinct EO profiles (Hussain et al., 2010). Myrcene (0.4-6.1%) and β-pinene (1.3-3.2%) are minor constituents but contribute to the overall aroma (Oliveira et al., 2022; Padalia et al., 2013).

Sesquiterpene hydrocarbons like trans-caryophyllene (0.1-20.3%) and germacrene D (0.2-23.4%) are prevalent in mint EO and contribute to its complex aroma (Spadaccino et al., 2021). β-Caryophyllene, found in concentrations ranging from 1.8% to 5.5%, adds to the EO's bioactivity (Shrigod et al., 2016).

In addition to these primary components, phenylpropanoids are present in small quantities, derived from the shikimate pathway (Ghasemzadeh et al., 2011). Thymol, though found in trace amounts, contributes to the medicinal properties of mint EO, particularly its antimicrobial activity (Mohammed et al., 2024). Mint essential oils (EOs) contain a wide range of compounds, with oxygenated monoterpenes like carvone and menthone being key contributors to aroma and flavor. Table 3. groups the main classes of chemical compounds.Monoterpene hydrocarbons, such as limonene, provide additional fragrance, though they are negatively correlated with oxygenated compounds. Sesquiterpene hydrocarbons, like trans-caryophyllene and germacrene D, further enhance the EO profile. Phenylpropanoids, though in small quantities, offer medicinal properties derived from the shikimate pathway. Minor compounds, like thymol, contribute to the therapeutic effects of mint EOs.

Tabel 3.Chemical composition of mint essential oil

Class	Component Name	Concentration Range (%)	Notable Observations	References
Oxygenated Monoterpenes	Carvone	0.1-73.4%	Major compound contributing to aroma and flavor.	Hussain et al., 2010
	Menthone	0.02-62%	Found in significant quantities in various landraces.	Eftekhari et al., 2021
	Neo-dihydrocarveol	0.08-54.7%	Varies significantly among landraces.	Shrigod et al., 2016
	1,8-Cineole (Eucalyptol)	0.1-46.28%	Negatively correlated with limonene and menthone.	Padalia et al., 2013
	Iso-Menthone	0.03-39.1%	Found in varying amounts in different landraces.	Arzani et al., 2005
	Pulegone	0.2-32.2%	Present in notable amounts in specific landraces.	Shrigod et al., 2016
	Menthol	Up to 3.02%	Found in specific samples.	Dambrauskienė et al., 2008
	Piperitenone Oxide	Up to 17.6%	High concentration in certain regional varieties.	Brada et al., 2007
	Piperitone	Up to 54.9%	Dominant in certain regional varieties.	Brada et al., 2007
	Linalool	Up to 86.7%	Significant aromatic contribution to EO profiles.	Mollaei et al., 2022
Monoterpene Hydrocarbons	Limonene	1.9-31%	Negatively correlated with carvone.	Hussain et al., 2010
	Myrcene	0.4-6.1%	Minor monoterpene hydrocarbon.	Oliveira et al., 2022
	β-Pinene	1.3-3.2%	Found in specific cultivars.	Padalia et al., 2013
Sesquiterpene Hydrocarbons	Trans-Caryophyllene	0.1-20.3%	Commonly found sesquiterpene hydrocarbon.	Spadaccino et al., 2021
	Germacrene D	0.2-23.4%	Major sesquiterpene hydrocarbon.	Spadaccino et al., 2021
	β-Caryophyllene	1.8-5.5%	Found in various samples.	Shrigod et al., 2016
Phenylpropanoids	-	Present in small quantities	Includes shikimate pathway-derived compounds.	Ghasemzadeh et al., 2011
Other Minor Compounds	Thymol	Variable	Found in traces, contributes to medicinal properties.	Mohammed et al., 2024

PROPERTIES OF MINT ESSENTIAL OIL

Mint EOs are renowned for their diverse range of bioactive properties, including antibacterial, antifungal, antioxidant, anti-inflammatory, and insecticidal effects, making them valuable in both therapeutic and agricultural applications.

Antioxidant activity

The antioxidant potential of *Mentha spicata* EO has been extensively studied, demonstrating significant free radical scavenging activity. In DPPH assays, the EO exhibited 25.14-63.39% inhibition at concentrations of 10-40 µg/mL, compared to ascorbic acid's 40.11-93.89% inhibition under similar conditions (Sangwan et al., 2001; Shah et al., 2024). The EO's antioxidant activity is attributed to its high levels of monoterpenes and oxygenated sesquiterpenes. *Mentha spicata* EO from South Tunisia, with menthone and pulegone as major components, exhibited an IC50 of 10 µg/mL, demonstrating even greater antioxidant potency (de Sousa et al., 2023). The antioxidant activity supports its potential use in food preservation, pharmaceuticals, and health supplements (López-Pedrouso et al., 2022).

Cancer and phytotherapy

EOs, with their diverse chemical compositions, including terpenes, phenols, and ketones, are emerging as promising complementary cancer therapies. For example, thymol and β-caryophyllene disrupt mitochondrial integrity and increase reactive oxygen species (ROS), leading to cell death. EOs from *Mentha arvensis* have shown cytotoxic effects against leukemia (Lampronti et al., 2006), and various Mentha species exhibit activity against breast cancer cells (Hussain et al., 2010). However, challenges such as bioavailability and selective toxicity limit their standalone use. Despite these challenges, EOs continue to show potential as adjuvants in cancer treatment, particularly when combined with chemotherapy drugs (Alabrahim et al. 2024)

Anti-inflammatory and cognitive benefits

Mentha spicata EO has demonstrated potent anti-inflammatory and antipyretic effects in Swiss NMRI mice (Desam et al., 2019). Additionally, spearmint extract has been shown to improve learning and memory in aging models, enhancing cognitive function (Zhang et al., 2021). Daily consumption of spearmint extract has been linked to improved attention and cognitive performance (Fernández-Lorente et al., 2019), with recent studies further confirming its positive effects on mood, sleep, and overall quality of life (Riaz et al., 2017).

Phytoremediation Potential

Mentha piperita has shown a strong capacity for phytoremediation, particularly in accumulating and translocating chromium species in contaminated soils. Studies indicate it can grow in fly ash-treated soil, accumulating various heavy metals in different plant parts without contaminating essential oils. Additionally, the ash from *Mentha piperita* can effectively remove Cr (VI) from water, offering a sustainable method for managing aquatic environments (Kedia et al., 2014).

Antifungal properties

Mint EOs, including those from *Mentha spicata*, *Mentha viridis*, and *Mentha piperita*, have proven effective as natural antifungal agents. Rich in bioactive compounds like carvone, menthol, menthone, and limonene, they disrupt fungal physiology and exhibit significant antifungal activity against various pathogens (Kedia et al., 2014).

Studies have shown *Mentha spicata* EO to be highly effective against *Aspergillus flavus*, a mold that produces aflatoxin B1 (Makkar et al., 2018). Mentha viridis EO demonstrated fungitoxicity against molds in stored corn, with its activity classified as fungicidal against several molds, including *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia lunata*, and *Mycelia sterilia*. Furthermore, mint EOs have shown effectiveness against yeast species like *Candida albicans* by disrupting biofilm formation and reducing fungal loads (Sameena et al., 2024). *Mentha piperita* EO has also proven effective against *Botrytis cinerea*, a pathogen responsible for significant agricultural losses, with enhanced stability and antifungal efficacy when incorporated into solid lipid nanoparticles.

Repellent and insecticidal properties

Mint essential oils (EOs), particularly from *Mentha piperita*, are also gaining attention for their insecticidal and repellent properties. Bioactive compounds such as terpenes, phenols, and aldehydes disrupt insect sensory systems, including olfactory receptors, preventing them from detecting attractants like human body odor and carbon dioxide (Kedia et al., 2014). *Mentha piperita* EO has been shown to disrupt the nervous system of insects, preventing them from approaching treated areas (Silva et al., 2021).

Fresh *Mentha piperita* EO emulsion exhibits 70-88% repellency against houseflies, though effectiveness diminishes over time. EO nanoparticles show high mortality rates in housefly larvae and are also effective against vector mosquitoes like *Anopheles stephensi* and *Aedes aegypti*. Additionally, the oil protects wheat grains from pests, demonstrating strong antifeedant activity. However, its efficacy is reduced when used with biological control agents like *Trichogramma pretiosum*, due to decreased female longevity.

Moreover, *Mentha spicata* EO has been found to inhibit oviposition in groundnut bruchid pests at a 2% concentration, indicating its potential as an eco-friendly repellent (Oliveira et al., 2022). Studies suggest that components of mint oils may mimic bruchid pheromones, further enhancing their application in sustainable agriculture (Fallah et al., 2024).

CONCLUSIONS

Mint essential oils are highly valued for their aromatic, therapeutic, and industrial applications, owing to their diverse chemical composition and bioactive properties. This review emphasizes the intricate relationship between sensory evaluation, physico-chemical analysis, and advanced instrumental techniques in assessing the quality and authenticity of mint essential oils. While traditional methods such as refractive index measurement, density determination, and acid value analysis remain crucial for quality control, their inability to detect subtle adulterations calls for the integration of more sophisticated analytical techniques.

Instrumental methods like GC, FTIR, and TLC have proven indispensable for identifying both volatile and non-volatile components, verifying oil authenticity, and detecting adulterants with high precision. The use of advanced chemometric models alongside ATR-FTIR spectroscopy provides rapid, non-destructive solutions for quality assessment, offering an effective bridge between laboratory research and practical applications. These cutting-edge technologies not only improve accuracy but also facilitate the development of portable devices, enabling on-site, field-level quality control.

However, challenges remain. Environmental factors, processing methods, and storage conditions continue to significantly affect the chemical composition and therapeutic properties of mint essential oils. To ensure consistency across different production scales and regions, there is a critical need for standardized analytical protocols and a wider adoption of modern techniques. Further research into non-volatile compounds and chiral analysis could provide deeper insights into the authenticity and functionality of these oils.

Sustainable cultivation and eco-friendly extraction practices, combined with advancements in green chemistry, will be key to meeting the growing demand for high-quality mint essential oils while preserving ecological balance.

In conclusion, the ongoing development of analytical techniques and interdisciplinary approaches will enhance the quality, authenticity, and potential applications of mint essential oils, solidifying their role in aromatherapy, pharmaceuticals, food, and cosmetics industries.

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NEW GENOTYPES OF COTTON (*GOSSYPIUM HIRSUTUM*) BRED AT BRGV BUZĂU

Matilda POPESCU[1], Costel VÎNĂTORU[1], Bianca MUȘAT[1], Camelia BRATU[1], Geanina NEGOȘANU[1], Adrian PETICILĂ[2]

[1]Plant Genetic Resources Bank Buzău, 56 Nicolae Bălcescu Blvd, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: hildepopescu@gmail.com

Abstract

Plant Genetic Resources Bank Buzău owns a rich collection of cotton (Gossypium hirsutum) varieties, predominantly ornamental cultivars, which are part of the institution's activity. Among these genotypes, two varieties with distinct phenotypic expressivity have been obtained as a result of intensive breeding work: L1, a cultivar with multiple uses, appreciated for its ornamental characteristics, and L2, which stood out for its earliness and high fiber production capacity. Biometric determinations revealed specific characteristics: L1 with an average height of 57 cm and mass/ boll 6.61 g and L2 with an average height of 109 cm and mass/ boll of 8.53 g. L1 was obtained from the crossing of the Romanian variety known as Brâncoveni and a local population from India and the L2 cultivar was based on the Adelin variety, one of the first cotton varieties in Romania. The present paper highlights the research carried out on the stabilization of two phenotypically distinct genotypes, to be approved and patented under the aegis of BRGV Buzau.

Key words*: biobanking, germplasm, phenotype, fiber, ornamental.*

INTRODUCTION

Cotton (*Gossypium* L.) belongs to the *Malvaceae* family and is one of the world's most important textile crops. It is grown in over 100 countries and meets about one third of global demand for textile fibers (Senchina et al., 2003). Management of cotton requires an understanding of the growth habit and responses of the plant to the environment (Whitaker et al., 2018). Throughout history, cotton has played a significant role in economic and social development, being used both in clothing and in other industries such as armaments and pulp (Townsend, 2020). In Romania, cotton cultivation began in 1868, initially on small areas of land in Moldova and Muntenia and later spread to the south of the country. Nowadays, cotton cultivation is facing challenges related to unfavorable climatic conditions, such as drought and high temperatures, which affect the quality and quantity of the fiber obtained (Majeed et al., 2021). Cotton is a perennial, semi-herbaceous species in its indigenous regions; however, in our country, it is grown as an annual crop. It develops a taproot that can extend up to 2 meters in depth, and the plant typically reaches a height ranging from 30 to 200 cm (Senchina et al., 2003).

The history of cotton cultivation is at least 3000 years old (Stewart et al. 2010). Cotton fiber quality is influenced by a complex interaction between genetics and environmental conditions (Bradow & Davidonis, 2000). Cotton is a crop that requires large auxiliary inputs and leaves a very low content of biomass residues on the soil after harvesting, compared to corn, wheat, and soybean (Trapalis et al., 2023).

Since the expression of different traits is often altered as breeding material due to environmental changes, cotton research must develop new, more productive cotton genotypes adapted to the current climate.

This paper presents research carried out at the BRGV Buzău on two cotton (*Gossypium hirsutum*) varieties: late-ripening L1 and early-ripening L2. The aim of the research was to evaluate the suitability of the two genotypes for introduction into cultivation in the S-E area under current climatic conditions.

MATERIALS AND METHODS

The research was carried out at BRGV Buzău, located in the SE part of Romania. The soil in this area (45°8′50″N 26°48′3″E/45.14722°N 26.80083°E), consist of loessosesoidal deposits, clays, marls and argillaceous marls (Soil map. Retrieved from http://geodim.meteoromania.ro/sia).

Groundwater is located at shallow depths ranging from 0.8 to 1 meter, although water in the surface crust layer tends to evaporate quickly, particularly during the heat of summer. The technology applied to the planted plots was that specific to cotton. In the fall, the soil was prepared through tillage to a depth of 30 cm. In spring, seedbed preparation was applied before sowing, which was carried out at the end of April, when the soil temperature reached 14°C. The sowing depth was 4-5 cm. The applied sowing pattern was 70 cm between rows and 40 cm plant spacing per row. Irrigation was carried out during the growing season as cotton is a species with low resistance to water stress.

The year 2024 was characterized by a pronounced drought during the peak period of plant growth and development, more precisely during the period of boll formation and maturation, with the phenomenon of heat stress. Phenological observations and biometric measurements were made according to UPOV (International Union for the Protection of New Varieties of Plants) and IPGRI (International Plant Genetic Resources Institute) requirements. The determinations were carried out in the experimental plots, on growing plants, and aimed at: measuring plant height, number of sympodial branches and number of capsules per plant. Capsule harvesting was staggered, from the base of the plants towards the top, as the ripening of the capsules is uneven and subject to genetic and climatic factors. Quality indices such as capsule weight, fiber/capsule mass and seed/capsule mass were determined, as well as biometric measurements of the seeds/capsule were made. Weighing was carried out in the Seed Quality Laboratory of BRGV Buzău using Kern five-decimal precision balance.

In order to valorize the production potential of the two lines, laboratory determinations of the physical quality assessment indices were carried out both at the fiber and seed level. In order to establish the vitality of the seeds as well as the vigor of the plants resulting from the harvested seeds, viability and germination determinations were made in Binder germination cabinets and the working parameters were monitored daily.

The methodology used was in accordance with ISTA (International Seed Testing Association) (2004) application standards. Viability testing was done to evaluate embryo structures and embryos in terms of their health, integrity and development, analyzing possible diseased, dry or immaturely developed seeds. For the viability test, from a 500 g seed homogenized sample, 200 seeds were extracted and divided into four replicates of 50 seeds each. These required prior preparation by removing, as far as possible, the felt brush, moistening in water for 18 hours, scarification by severing the integument at the distal end of the cotyledons, and longitudinally severing the seeds ¾ through the central axis. After preparation, the seeds were placed in a 1% concentration of 2,3,5-triphenyl tetrazolium (TTZ) solution in order to highlight viable tissues by staining for 3 hours. Evaluation was performed according to international standards (International Seed Testing Association, 2024).

Germination testing was carried out according to ISTA 2024 standards to determine the percentage of seeds capable of producing normal germination.

For the germination test, 200 seeds were placed equidistantly with sufficient space for growth, on two layers of blotting paper. The filter paper, with 80g/ml mass and 16.7 x 50.8 cm dimensions, was sterilized and moistened to 60% of the holding capacity for water. The pH of the water was neutral (7.28), as required for seed germination. The germination temperature was alternating 8 h, at 20°C and 16 h, at 30°C in the presence of light. The relative humidity inside the cabinet was 85%. Essential structures were subjected to evaluation when they were sufficiently well developed.

The genetic resources analyzed were two cotton varieties of the *Gossypium hirsutum* species. Variety L1 is characterized by late maturity, has ornamental importance and is ideal for commercialization of dried open caps stems andfor flower arrangements. It is also characterized by dark brown flowering stem, decorative, intensely coloured flowers (Figure 1 a) and very well encapsulated fibers, which do not deteriorate over a long period of time.

Variety L2 belongs to the early maturity group and is characterized by high fiber production, slightly dehiscent capsules that open wide, thus facilitating fiber harvesting (Figure 1 b).



a) b)

Figure 1. Cotton batches in forming phenophase:
a) L1 genotype; b) L2 genotype

In the full maturity phenophase, three successive measurements were made at 5-day intervals, and the mean value and standard deviation were calculated. Analysis of variance (Anova test) was performed to determine significant differences between the two genotypes.

RESULTS AND DISCUSSIONS

The vegetation time of the plants was 143 days and the developmental phenophase from the beginning of the flowering to the last boll harvest was of 85 days (Figure 2).
Both genotypes were characterized by an erect main stem, circular in cross-section. Plants formed at the base of the stem, 2-3 vegetative branches of flowers which were removed.



a) b)

Figure 2 a) Cotton flower; b) evolution of cotton flower after fertilization until boll maturation

The fruiting (sympodial) branches had a flower at each internode. Under 2024 weather conditions, cotton flowering lasted until the end of October (Figure 3).



Figure 3. Cotton bolls at different stages of ripening

The statistical analysis performed revealed significant differences between the two lines in plant height and number of seeds/capsule ($p \leq 0.001$) (Table 1), which can be attributed to genetic variation in their chromosomal map.

Table1. Biometric determinations on the two cotton lines L1 and L2

Geno type	pH (cm) Mean ± SD	NSB Mean ± SD	TNB Mean ± SD	NC/P Mean ± SD	MC (g) Mean ± SD	FL (cm) Mean ± SD	NS/C Mean ± SD
L1	62.8± 5.63	16.9± 2	29.6± 3.84	21.14 ±3.07	7.11± 0.93	3±0.3 8	18.4± 2.88
L2	109.2 ±2.77	21.8± 2.38	31.2± 1.92	25.8± 2.38	8.56± 0.20	3.56± 0.20	26.8± 2.38
p ≤ 0.001	<0.00 1	0.008	0.43	0.028	0.01	0.02	0.001

Legend: PH - plant height; NSB - Number of sympodial branches; TNB - Total number of branches; NC/P - Number of capsules/plant; MC - Mass of capsules; FL - Fiber length; NS/C - Number of seeds/ capsules.

The average length of the fibers extracted from the analyzed bolls showed that L2 was 3.5 cm compared to L1, which had a value of 2.7 cm. In terms of the number of seeds in the boll, L2 had an average of 26 seeds/boll compared to L1 of only 18 seeds/boll (Figure 4).

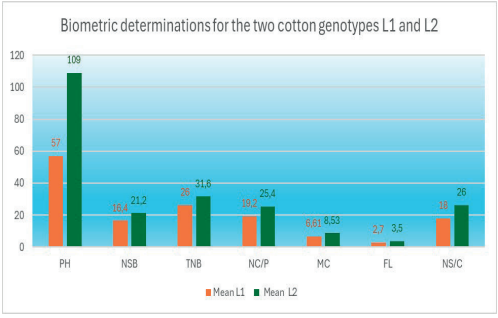


Figure 4. Graphical representation of the results of the biometric measurements on the two cotton lines L1 and L2

The analysis of the qualitative characteristics carried out aimed, in the first place, to identify the genotype with superior quality fiber, cotton being a technical plant intended for fiber production. The measurements performed on 100 bolls revealed that L2 has a 29.30% fiber/boll mass ratio compared to L1 with a ratio of 25% fiber/boll mass. L2 also showed a superior fiber quality in terms of organoleptic characteristics (Table 2).

Table 2. Qualitative determinations of cotton fibers obtained from batches L1 and L2

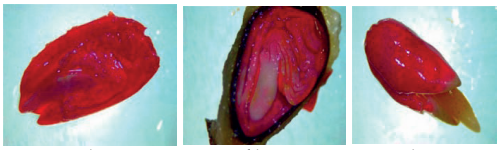
Determinations	L1 (late variant)	L2 (early variant)
Mass fiber/capsule %	25%	29.30 %
Thread smoothness	matte	silky
Colour	white yellowish	white, silky
Linter	short, gray	silky white, with adherence to the integument

By analyzing the two lines, the L2 variety was noted with an average seed weight of 5.1 g compared to 4.9 g recorded for L1. Qualitative characteristics such as seed shape and colour are identical in the two genotypes and the ratio of seed coat to seed mass was highest in L2 (22%) compared to L1 (21%). The maximum level of seed length was recorded in L2 cultivar, i.e 9.1 mm, the same for width, i.e 4.8 mm (Table 3).

Tabel 3. Biometric seed measurements of L1 and L2 cotton varieties

Determinations	L1 Mean/100 seeds	L2 Mean/100 seeds
Average mass/seed (g)	4.9	5.1
Shape	elongated oval	elongated oval
Colour	dark brown	dark brown
Mass ratio husk/seed	21%	22%
Seed coat thickness (mm)	0.25 mm	0.22 mm
Embryonic mass	80%	80%
Average length (mm)	8.5 mm	9.1 mm
Average width (mm)	4.2 mm	4.8 mm

Viability testing was carried out conforming to the assessment criteria (ISTA, 2024) according to which fully coloured seeds or seeds with small uncoloured areas of essential structures are considered viable and seeds showing uncoloured essential embryo structures or uncoloured essential areas of essential structures or uncoloured embryo as a whole are considered non-viable, according to the international assessment schemes (Tetrazolium Testing - International Seed Testing Association, 2003). After 3 hours of coloring, each seed was evaluated individually, L-2 showing a better uniformity between replications, the difference between minimum and maximum being only 4% (R1 and R3) (Figure 5).



a) b) c)

Figure 5. Viability assessment structures evaluated: a) viable embryos in section; b) viable embryos in tegument; c) non-viable embryo

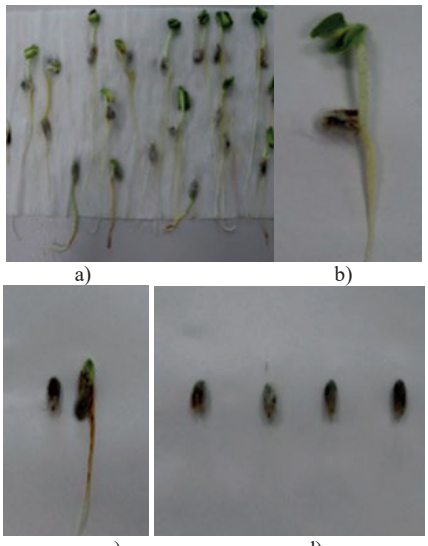
The uniformity of the coloration of the evaluated structure parts of each seed was also evident. Viability recorded a maximum value of 95% in L2 and 90% in L1. The non-viable seeds showed embryos totally uncoloured or with essential parts of the basic structures uncoloured. L1 revealed 10% non-viable seeds, while L2, 5% non-viable seeds (Table 4).

Table 4. Viability test evaluation in L1 and L2 cotton varieties

Replications	L1 (late variant)		L2 (early variant)	
	Viable seeds	Non-viable seeds	Viable seeds	Non-viable seeds
R1	46	4	48	2
R2	45	4	48	2
R3	48	4	46	4
R4	42	8	47	3
Total	180	20	189	11
%	90	10	95	5
Means	45.25	5	47.25	2.75
Variant calculation	6.25	4	0.9167	0.167

Following L1 evaluations, normal sprouts showed a development characteristic of the species for this time of evaluation (germination energy) with high vigour and good growth uniformity (Figure 6 a), a strong taproot, well

developed hypocotyl, characteristic cotyledons with a true leaf-like, crumpled appearance (Figure 6 b). The abnormal germs showed primary infection on all structures that did not allow their further development in normal plants (Figure 6 c) which led to the appearance of dead seeds (Figure 6 d).



a) b) c) d)

Figure 6. L1 germination evaluation:
a) germination layer detail; b) normal germ;
c) abnormal germ; d) dead seeds

It was found that L2 (the early variety) showed the maximum germination value of 94% and L1 (the late variety), 89% germination value. It was found to be a 3% abnormal germplasm for both genotypes, cotton showing a higher susceptibility to attack by seed-borne diseases. In case of dead seed evaluation, the maximum level was identified in L1, i.e 8% and 3%, in L2 (Table 5).

Table 5. Germination evaluation of the 2 cotton varieties L-1 and L-2

Replications	L1 (late variant)				L2 (early variant)			
	GN	GA	DS	FS	GN	GA	DS	FS
R1	45	2	3	0	47	1	2	0
R2	42	3	5	0	45	2	3	0
R3	46	1	3	0	48	1	1	0
R4	44	1	5	0	48	1	1	0
total	177	7	16	0	188	5	7	0
%	89	3	8	0	94	3	3	0
Mean	44.25	1.75	4.00	0	47.5	1.25	1.75	0

Legend: GN-normal germ; GA-abnormal germ; DS- dead seed; FS-fresh seed.

The analysis of the compatibility between viability and germination highlighted that L2 recorded an average value of 95%, while L1 recorded 90%. The graphical interpretation confirms the superior quality of the L2 genotype (Figure 7).

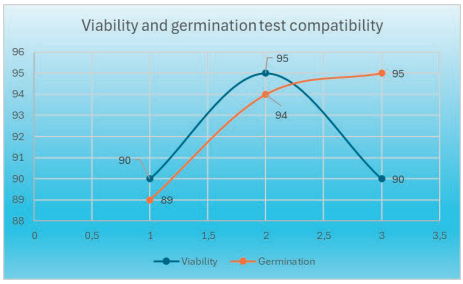


Figure 7. The graphical representation of the compatibility of the results from the two tests (viability and germination) for the two varieties L1 (late) - L2 (early)

CONCLUSIONS

The results obtained confirmed the significant phenotypic and technical differences between the two cultivars.

The breeding research conducted highlighted genotype L2 as having superior quantitative and qualitative characteristics compared to genotype L1. At the same time, the studies undertaken at BRGV Buzău confirmed the acclimatization potential of both genotypes, which can be successfully cultivated under the climatic conditions of the Buzău area.

L1 presents a great ornamental potential and can be cultivated for this purpose. The acclimatized and genetically stabilized genotypes will be submitted for homologation and patenting under the auspices of BRGV Buzău.

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MICROPROPAGATION OF *THYMUS* x *CITRIODORUS* AND GENETIC UNIFORMITY ASSESSMENT OF *IN VITRO* REGENERATED PLANTS

Ana-Maria RADOMIR[1], Ramona STAN[1], Doina CLAPA[2], Adrian PETICILĂ[3], Andreea Elena MANOLESCU[1], Dorin Ioan SUMEDREA[1]

[1]National Research and Development Institute for Biotechnology in Horticulture Stefăneşti-Argeş, 37 Bucharest-Piteşti Road, 117715, Stefăneşti, Argeş, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur, 400372, Cluj-Napoca, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: andreeamanolescu16@gmail.com

Abstract

Thymus x citriodorus (Pers.) Schreb. (lemon thyme) is a perennial aromatic plant valued for its culinary, medicinal, and ornamental properties. This study aimed to establish an efficient micropropagation protocol for lemon thyme and to evaluate the genetic fidelity of in vitro regenerated plants using RAPD molecular markers. The highest shoot multiplication rate (13.77±2.51 shoots/explant) was obtained on Murashige and Skoog (MS) medium containing 3 mg/L BAP. The longest shoots (3.76±0.46 cm) were generated in the presence of 0.5 mg/L 2iP and 5 mg/L GA_3. Rooting was most efficient (100% rooting rate) on ½MS medium supplemented with IBA (0.5 or 1 mg/L) and in the presence of IBA (0.5 or 1 mg/L) in combination with GA_3 (3 mg/L). Acclimatized plants showed a 100% survival rate. Molecular analyses revealed monomorphic banding patterns in RAPD profiles, confirming the genetic fidelity of micropropagated plants.

Key words: *lemon thyme, micropropagation, plant growth regulators, genetic fidelity, RAPD markers.*

INTRODUCTION

Thymus x citriodorus (Pers.) Schreb (lemon thyme) is a perennial species of the *Lamiaceae* family, well known for its strong lemon-like aroma and high content of essential oils, which possess antimicrobial, antifungal, and antioxidant properties (Oliveira et al., 2022; Oliveira et al., 2023; Tevfik and Yegorova, 2024). This plant is widely used in the food, cosmetic, and pharmaceutical industries, as well as in landscaping, being a popular ornamental and melliferous species (Zheljazkov et al., 2008).

Conventional propagation methods, such as stem cuttings or clump division, are limited by the species' slow growth rate and low ability to multiply. Moreover, propagation by seeds is often inefficient due to the low viability and high genetic variability of regenerated plants (Kintzios, 2000). In this context, *in vitro* culture techniques offer a promising alternative for the rapid and large-scale production of healthy and genetically uniform plant material (George et al., 2008). Micropropagation is a valuable method for preserving and multiplying elite genotypes; however, it can also induce somaclonal variations due to physiological stress under *in vitro* conditions (Jain, 2001). Therefore, assessing the genetic stability of *in vitro* regenerated plants is essential for their subsequent use in commercial production or research (Kudikala et al., 2020; Biswas et al., 2022; Mudoi et al., 2023). DNA molecular markers, such as RAPD, ISSR, or SSR, have proven effective in detecting genetic variation and validating clonal fidelity (Rani and Raina, 2000; Bairu et al., 2011).

This study aimed to develop an efficient micropropagation protocol for *T. x citriodorus* and to evaluate the genetic uniformity of regenerated plants using RAPD molecular markers. The results will contribute to optimizing *in vitro* propagation strategies and ensuring the genetic quality of plant material intended for commercial and therapeutic use.

MATERIALS AND METHODS

In vitro propagation

The *in vitro* propagation technique involves four essential stages: culture initiation, shoot multiplication, shoot rooting, and acclimatization of regenerated plantlets to *ex vitro* conditions.

In vitro culture initiation stage

The explants used for initiating *in vitro* cultures consisted of apexes and uninodal fragments taken from shoots harvested from *T. x citriodorus* mother plants selected from a phytosanitary point of view and for the authenticity of the variety.

The shoots were defoliated, fragmented, and first washed with tap water, after which they were sterilized with calcium hypochlorite ($CaCl_2O_2$) in different concentrations: 6%, 8%, and 10%. The sterilization time was 10 minutes. Subsequently, three rinses with sterile distilled water were performed to remove traces of sterilizing agent.

After disinfection of the plant material, the explants were sampled and inoculated under aseptic conditions on MS culture medium (Murashige and Skoog, 1962) without plant growth regulators (PGRs).

At this stage of the experiment, as well as in subsequent stages, the culture medium was supplemented with 40 g/L glucose, 32 mg/L NaFeEDTA, and 7 g/L agar.

The culture media were sterilized by autoclaving at 120°C for 20 min. Before autoclaving, the pH of the medium was adjusted to 5.6-5.8 with 1N KOH or 1N HCl.

The cultures were maintained in the growth chamber under controlled conditions of temperature (22-24ºC), photoperiod (16 hours light/8 hours dark), and light intensity (2500 lx).

Four weeks after culture initiation, the following parameters were calculated: the percentage of infected explants (explants contaminated with fungal and/or bacterial infections), the percentage of necrotic explants (uncontaminated but non-viable explants, burned by the disinfectant agent), and the percentage of initiation (viable explants that regenerated shoots).

The experiments were organized in 3 repetitions. For each experimental variant and each repetition, 10 explants were used.

In vitro multiplication stage

The apexes and nodal fragments resulting from the division of regenerated shoots during the *in vitro* culture initiation phase were inoculated on MS medium supplemented with different types, combinations, and concentrations of PGRs. MS medium without PGRs was used as control medium (Table 1).

Table 1. Experimental variants - *in vitro* multiplication of *T. x citriodorus* shoots

Variant	PGRs (mg/L)				
	BAP	KIN	2iP	IAA	GA_3
V0	-	-	-	-	-
V1	0.5	-	-	-	-
V2	3	-	-	-	-
V3	5	-	-	-	-
V4	-	0.5	-	-	-
V5	-	3	-	-	-
V6	-	5	-	-	-
V7	-	-	0.5	-	-
V8	-	-	3	-	-
V9	-	-	5	-	-
V10	0.5	-	-	1	-
V11	3	-	-	1	-
V12	5	-	-	1	-
V13	-	0.5	-	1	-
V14	-	3	-	1	-
V15	-	5	-	1	-
V16	-	-	0.5	1	-
V17	-	-	3	1	-
V18	-	-	5	1	-
V19	-	-	-	-	0.5
V20	-	-	-	-	3
V21	-	-	-	-	5
V22	0.5	-	-	-	0.5
V23	3	-	-	-	0.5
V24	5	-	-	-	0.5
V25	0.5	-	-	-	3
V26	3	-	-	-	3
V27	5	-	-	-	3
V28	0.5	-	-	-	5
V29	3	-	-	-	5
V30	5	-	-	-	5
V31	-	0.5	-	-	0.5
V32	-	3	-	-	0.5
V33	-	5	-	-	0.5
V34	-	0.5	-	-	3
V35	-	3	-	-	3
V36	-	5	-	-	3
V37	-	0.5	-	-	5
V38	-	3	-	-	5
V39	-	5	-	-	5
V40	-	-	0.5	-	0.5
V41	-	-	3	-	0.5
V42	-	-	5	-	0.5
V43	-	-	0.5	-	3
V44	-	-	3	-	3
V45	-	-	5	-	3
V46	-	-	0.5	-	5
V47	-	-	3	-	5
V48	-	-	5	-	5

BAP - 6-benzylaminopurine; KIN - kinetin; 2iP - 2-isopentyl adenine; IAA - indole-3-acetic acid; GA_3 - gibberellic acid.

Subculturing of biological material on fresh medium was performed every four weeks.
Four weeks after subcultivation, the number of shoots per explant and shoot length were evaluated.
The experiments were organized in 3 repetitions. For each experimental variant and each repetition, 10 explants were used.
To evaluate the *in vitro* regenerative potential of the studied species in successive subcultures, the regenerated biological material was cultured on MS medium supplemented with 40 g/L glucose, 32 mg/L NaFeEDTA, 7 g/L agar, and 3 mg/L BAP. Three successive subcultures were performed every four weeks. The parameters evaluated after each of the three subcultures were the number of shoots per explant and shoot length.

In vitro rooting stage
Shoots regenerated on the propagation medium were individualized and cultivated on several variants of rooting medium. ½MS medium without PGRs was used as control medium (Table 2).

Table 2. Experimental variants - *in vitro* rooting of *T. x citriodorus* shoots

Variant	PGRs (mg/L)			
	IBA	NAA	IAA	GA_3
V0	-	-	-	-
V1	0.5	-	-	-
V2	1	-	-	-
V3	-	0.5	-	-
V4	-	1	-	-
V5	-	-	0.5	-
V6	-	-	1	-
V7	0.5	-	-	3
V8	1	-	-	3
V9	-	0.5	-	3
V10	-	1	-	3
V11	-	-	0.5	3
V12	-	-	1	3

IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid; IAA - indole-3-acetic acid; GA_3 - gibberellic acid.

Four weeks after the initiation of the experiment, the rooting rate was calculated.
The experiments were organized in 3 repetitions. For each experimental variant and each repetition, 10 shoots were used.

Acclimatization stage
In vitro rooted shoots were carefully removed from the culture vessels, and their root system was rinsed thoroughly under running tap water to remove residual agar medium and reduce the risk of contamination with phytopathogenic microorganisms. They were subsequently transplanted into a substrate composed of peat, manure, and perlite (in a ratio of 2:1:1) and Jiffy peat pellets.
The plants were placed under glass jars or covered with plastic bags to create a high-humidity environment and prevent dehydration. Two to three weeks after transfer to *ex vitro* conditions, when the plants began to grow, the humidity was gradually reduced by uncovering the cultures, allowing the plants to adapt to natural environmental conditions. The appearance of the first *ex vitro* leaves signaled the end of the acclimatization phase.
Four weeks after the start of the experiment, the acclimatization rate was calculated.
The experiments were organized in 3 repetitions. For each experimental variant and each repetition, 30 plantlets were used.
The acclimatized plants were then transplanted into pots in a peat-based mixture for further fortification and development.

Genetic uniformity assessment of *in vitro* regenerated plants
The genetic fidelity of *in vitro* propagated *T. x citriodorus* plants was assessed using Random Amplified Polymorphic DNA (RAPD) markers. The biological material for genetic analyses consisted of young leaves of ten *T. x citriodorus* plants: the mother plant, four *in vitro* plants, and five acclimatized plants.

Genomic DNA extraction
Genomic DNA was extracted using the Qiagen DNeasy Plant Mini kit, following the manufacturer's protocol with minor adjustments.

DNA quality assessment
The concentration and purity of extracted DNA were determined spectrophotometrically using a BioPhotometer plus (Eppendorf, Germany).

RAPD amplification
For the RAPD analysis, a total of sixteen primers from the OPA and OPB series (OPA-09 to OPA-20 and OPB-01 to OPB-04) were selected based on their proven ability to generate polymorphic and reproducible banding patterns (Kim et al., 2020).
PCR reactions were performed in a final volume of 25 µL, containing 5 µL of 5×

FirePol Master Mix Ready to Load (Solis BioDyne, Estonia), 3 µL of genomic DNA (~10 ng), 2 µL of primer, and ultrapure water. Amplifications were performed in a Techne TC-512 Thermal Cycler under the following conditions: an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 20 s, annealing at 35°C for 40 s, and extension at 72°C for 60 s, with a final extension of 5 min at 72°C.

Electrophoretic analysis

Amplified DNA fragments were separated by electrophoresis in 1.2% agarose gels prepared in 1× TAE buffer and stained with ethidium bromide. Visualization was carried out under UV illumination using the Gene Flash Syngene Bio Imaging system.

Data analysis

Statistical interpretation of data was performed using SPSS 10 for Windows program. The differences between the mean values of the analyzed morphological characteristics of *in vitro* plants grown on nutrient media with different hormonal formulas compared to the control were analyzed with One-way ANOVA – LSD, being considered significant at $P<0.05$. Values shown are means ± standard deviation (SD).

RESULTS AND DISCUSSIONS

***In vitro* propagation**

To develop an efficient *in vitro* propagation protocol for *T. x citriodorus*, the optimal conditions were identified for each regeneration stage.

In vitro culture initiation stage

One of the essential conditions on which the success of initiating a cell culture depends is the selection of the appropriate sterilization method for disinfecting the explants.

In this experiment, the inclusion of *T. x citriodorus* species in the *in vitro* culture system did not raise any particular problems, the use of $CaCl_2O_2$ for sterilizing the explants proving effective.

Between 66.67% and 83.33% of the explants regenerated shoots. The highest initiation percentage was obtained at the lowest concentration of the sterilizing agent, namely 6%. As the concentration of the disinfectant agent increased, a decrease in the initiation rate was observed, with the lowest values being obtained at a concentration of 10% $CaCl_2O_2$ (Figure 1).

The percentage of infected explants after the first four weeks of culture ranged between 6.67% and 13.33%. The lowest contamination rate was recorded when using 10% $CaCl_2O_2$, but in this case, the percentage of necrotic explants that did not regenerate shoots was higher compared to that recorded when using 6% or 8% $CaCl_2O_2$ (Figure 1).

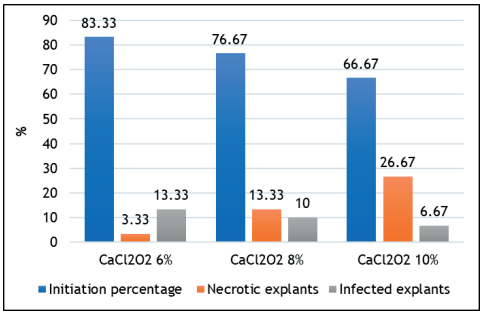


Figure 1. Influence of sterilization method on the *in vitro* regenerative potential of *T. x citriodorus* explants at the culture initiation stage. Values shown are means of 3 repetitions.

Considering that when $CaCl_2O_2$ at a concentration of 6% was used as a disinfectant, the explants had the best regenerative potential, we can conclude that this sterilization method was the most effective.

The use of MS basal medium without PGRs favored the production of plantlets that were subsequently used to test the morphogenetic response of *T. x citriodorus* explants on nutrient media with different hormonal formulas (Figures 6 a and 6 b).

In contrast to our results, a low survival rate of initial explants has been reported in other *Thymus* species, such as *T. mastichina* (Mendes and Romano, 1999), *T. caespititius* (Mendes et al., 2013), and *T. moroderi* (Marco-Medina and Casas, 2015).

In vitro multiplication stage

The highest proliferation rate in *in vitro* culture usually requires the search for an appropriate phytohormonal balance.

In our study, supplementing the nutrient

medium with PGRs induced an increase compared to the control medium (without PGRs) in the number of shoots per explant, in most variants the increases being significant. The highest values (13.77±2.51 shoots/explant) were obtained on MS medium supplemented with 3 mg/L BAP, followed by culture medium supplemented with 5 mg/L BAP and 5 mg/L GA_3 (10.53±0.85 shoots/explant). The lowest multiplication rate values were recorded in the control variant lacking PGRs (1.13±0.06 shoots/explant) (Table 3). In comparison, the study of the influence of culture medium composition on the morphometric parameters of *T.* × *citriodorus* microshoots, conducted by Tevfik and Yegorova (2024), revealed that the maximum number of shoots (8.6 shoots/explant) was obtained on MS medium containing 1.0 mg/L BAP and 1.0 mg/L GA_3. Clapa et al. (2025) obtained 29.8±1.26 shoots per explant in *T. x citriodorus* on Driver and Kuniyaki Walnut (DKW) culture medium (Driver and Kuniyuki, 1984) supplemented with 0.5 mg/L BA. In *T. marschallianus*, an increase in this parameter was observed when MS culture medium supplemented with 1.0 mg/L TDZ and 1.0 mg/L GA_3 or 0.5 mg/L IAA was used (Tevfik and Yegorova, 2024). *T. caespititius* was optimally multiplied in the presence of 1 mg/L BA and 0.25 mg/L IBA (Mendes et al., 2013). The results obtained by Kulpa et al. (2018) indicated that the cytokinin that had the most positive impact on shoot development of *T. vulgaris* L. at the multiplication stage was 2iP (at a concentration of 5 mg/L). In the case of the species *T. vulgaris* and *T. longicaulis*, the optimized proliferation medium consisted of semi-solid MS medium supplemented with 1 mg/L kinetin and 0.3 mg/L GA_3 (Ozudogru et al., 2011). In *T. lotocephalus*, BA gave the best proliferation results, but also high percentages of hyperhydric shoots (Coelho et al., 2012). In research conducted by Nordine and El Meskaoui (Nordine and El Meskaoui, 2014) on *T. broussonetii*, the maximum number of shoots was obtained on a medium supplemented with 0.5 mg/L BAP and 0.2 mg/L NAA, but the developed shoots were short.

Regarding shoot length, in our study, the shortest shoots were recorded on the culture medium supplemented with 5 mg/L BAP (0.79±0.07 cm), and the longest shoots were generated in the presence of 0.5 mg/L 2iP and 5 mg/L GA_3, respectively 3.87±0.66 cm and 3 mg/L GA_3 with an average of 3.76±0.46 cm (Table 3).

Table 3. Influence of type, concentration, and combination of PGRs on the number of shoots/explant and shoot length in the species *T. x citriodorus*. Values shown are means ± SD

Variant	No. of shoots/ explant	Shoot length (cm)
V0	1.13±0.06	1.68±0.04
V1	4.53±1.12*	1.28±0.09*
V2	13.77±2.51*	1.18±0.06*
V3	3.17±0.64*	0.79±0.07*
V4	4.03±0.35*	1.11±0.02*
V5	2.87±0.86*	0.90±0.03*
V6	3.93±0.35*	0.92±0.07*
V7	1.57±0.21	1.33±0.22*
V8	3.03±0.55*	1.05±0.03*
V9	2.47±0.15*	0.91±0.08*
V10	5.07±0.23*	0.93±0.07*
V11	4.90±0.87*	0.81±0.08*
V12	5.67±0.87*	0.82±0.03*
V13	2.60±0.20*	1.20±0.09*
V14	2.90±0.20*	0.84±0.07*
V15	3.03±0.55*	0.80±0.05*
V16	1.73±0.25	1.50±0.23
V17	5.00±0.70*	1.18±0.03*
V18	4.67±0.96*	0.93±0.09*
V19	1.13±0.12	2.47±0.53*
V20	1.23±0.15	3.76±0.46*
V21	1.70±0.46	3.20±0.29*
V22	4.17±0.57*	1.20±0.13*
V23	4.37±0.25*	1.08±0.04*
V24	4.27±0.21*	1.10±0.04*
V25	4.40±0.26*	1.55±0.16
V26	4.17±0.15*	1.35±0.13*
V27	4.70±0.35*	1.14±0.03*
V28	5.00±1.22*	1.35±0.12*
V29	5.23±0.61*	1.25±0.09*
V30	10.53±0.85*	1.29±0.03*
V31	2.53±0.61*	3.53±0.46*
V32	2.93±0.23*	1.14±0.03*
V33	2.87±0.15*	1.09±0.09*
V34	3.50±0.17*	2.10±0.02*
V35	3.40±0.44*	1.43±0.20
V36	3.80±0.36*	1.42±0.16
V37	3.63±0.06*	2.60±0.10*
V38	3.43±0.45*	1.72±0.16
V39	3.87±0.64*	1.67±0.09
V40	2.23±0.25*	2.32±0.22*
V41	3.03±0.49*	1.40±0.19
V42	2.83±0.31*	1.34±0.11*
V43	1.63±0.12	2.99±0.22*
V44	3.17±0.21*	1.98±0.26
V45	2.33±0.21*	1.96±0.08
V46	1.77±0.15	3.87±0.66*
V47	2.50±0.40*	2.36±0.32*
V48	2.57±0.40*	2.18±0.11*

*significance of the difference at $p < 0.05$

Research conducted by Tevfik and Yegorova (2024) on the dependence of *T.* × *citriodorus* shoot length on the type, concentration, and combination of PGRs in the culture medium showed that this parameter reached the highest values when MS hormone-free medium (1.6±0.2 cm) or MS medium supplemented with 1.0 mg/L Kin and 1.0 mg/L GA_3 (1.3±0.1 cm) was used. Clapa et al. (2025) obtained shoots with a length of 2.99±0.07 cm in *T. x citriodorus* on DKW culture medium supplemented with 0.5 mg/L BA. In *T. marschallianus*, the longest shoots were observed on medium supplemented with 1.0 mg/L Kin and 1.0 mg/L BAP (1.0±0.1 cm) (Tevfik and Yegorova, 2024). According to Nordine and El Meskaoui (Nordine and El Meskaoui, 2014), multiplication of thyme on media supplemented with cytokinins resulted in a decrease in shoot length.

In our study, on culture media supplemented with 0.5 mg/L 2iP and 1 mg/L IAA (V16), 0.5 mg/L GA_3 (V19), 3 mg/L GA_3 (V20), and 5 mg/L GA_3 (V21), regenerated shoots also formed roots. The rhizogenesis process also occurred in the control variant lacking PGRs (V0).

The *in vitro* regenerative potential of the studied species remained approximately constant throughout the three subcultures performed. There were no statistically significant differences between the three subcultures in terms of the number of shoots/explant (Figure 2) and shoot length (Figure 3).

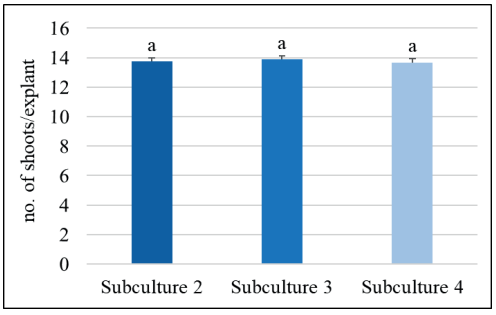


Figure 2. Influence of the number of subcultures on the number of shoots/explant in *T. x citriodorus*. The mean values are accompanied by error bars indicating standard deviations. Letters indicate the significance of differences between subcultures according to the LSD test ($P < 0.05$)

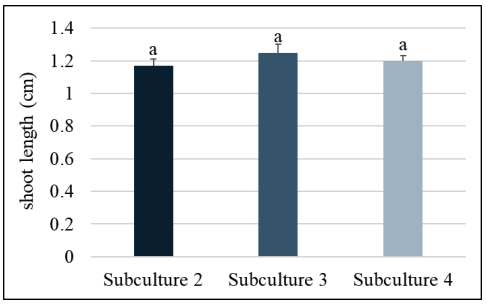


Figure 3. Influence of the number of subcultures on shoot length in *T. x citriodorus*. The mean values are accompanied by error bars indicating standard deviations. Letters indicate the significance of differences between subcultures according to the LSD test ($P < 0.05$)

From a qualitative point of view, the biological material regenerated *in vitro* had normal morphology, without aspects of vitrification, necrosis, or callus differentiation (Figures 6 c and 6 d).

***In vitro* rooting stage**

For effective *in vitro* propagation protocols, shoots must produce roots in large proportions. In the absence of roots, the survival rate of acclimatized plants is reduced.

It is known that, in *in vitro* cultures, auxins are responsible for stimulating root development and cell elongation. However, high concentrations of auxins added to nutrient media result in callus development and inhibition of root system development (Yankovskaya et al., 2011).

Thus, to select the optimal composition of the nutrient medium for the rooting of *T. x citriodorus* shoots, several medium variants were tested in the present study, in which the type of auxin (IBA, NAA, IAA) and its concentration (0.5 and 1 mg/L) were varied. The combination of auxin with gibberellic acid was also tested.

The highest values of rooting rate (100%) were obtained when the culture medium was supplemented with IBA (0.5 or 1 mg/L). A rooting rate of 100% was also obtained in the presence of IBA (0.5 or 1 mg/L) in combination with GA_3 (3 mg/L) (Figure 4).

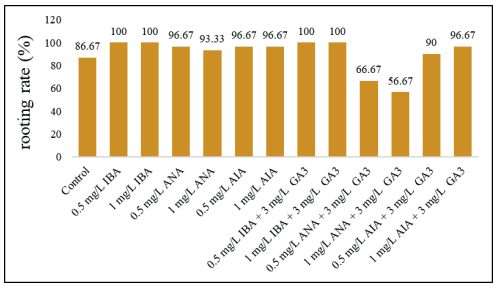


Figure 4. Influence of type, concentration, and combination of PGRs on the *in vitro* rooting capacity of *T. x citriodorus* shoots. Values shown are means of 3 repetitions

Similar results were reported by Kulpa et al. (2018) for *T. vulgaris*, the most developed root system being obtained on medium supplemented with 1 mg/L IBA. In contrast, Ozudogru et al. (2011) reported that the most effective medium for *in vitro* rooting of thyme shoots was MS medium supplemented with 0.01 mg/L NAA. Research conducted by Coelho et al. (2012) demonstrated that the best results in the rooting stage were obtained on ¼MS medium without auxins or supplemented with 0.5 mg/L IAA. The rooting percentage obtained by Clapa et al. (2025) for *T. x citriodorus* on DKW medium without PGRs was 71.67%. According to Nordine and El Meskaoui (2014) and Nordine et al. (2013), the most suitable medium for rooting of *Thymus* sp. shoots was MS medium without PGRs.

Acclimatization stage

The acclimatization is the final stage of microclonal propagation. For efficient micropropagation protocols, it is essential that *in vitro* regenerated plants successfully acclimate to *ex vitro* conditions.

In our experiments conducted on *T. x citriodorus*, an acclimatization rate of 100% was recorded in both nutrient substrates used: Jiffy peat pellets and a mixture of peat, manure, and perlite (in a ratio of 2:1:1) (Figure 5).

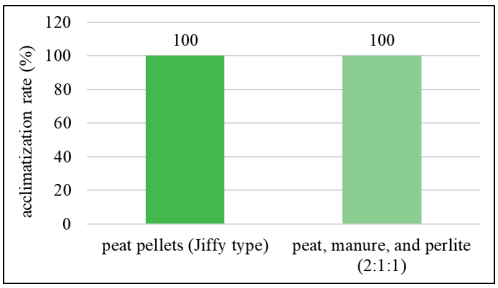


Figure 5. Influence of nutrient substrate on the acclimatization capacity of *in vitro* rooted plants to *ex vitro* conditions. Values shown are means of 3 repetitions

Similar results were obtained by Clapa et al. (2025) when acclimatizing *in vitro* rooted shoots of *T. x citriodorus* after *ex vitro* transfer to perlite.

Successful acclimatization of *in vitro* regenerated plantlets to *ex vitro* conditions was also achieved in other thyme species: *T. vulgaris* (a survival rate of 100%) (Ozudogru et al., 2011), *T. bleicherianus* (a survival rate of 85%) (Nordine and El Meskaoui, 2014), *T. hyemalis* (a survival rate of 90%) (Nordine et al., 2013), and *T. lotocephalus* (a survival rate of 93.33%) (Coelho et al., 2012).

Figure 6 shows aspects of the main stages of *in vitro* culture of *T. x citriodorus*.

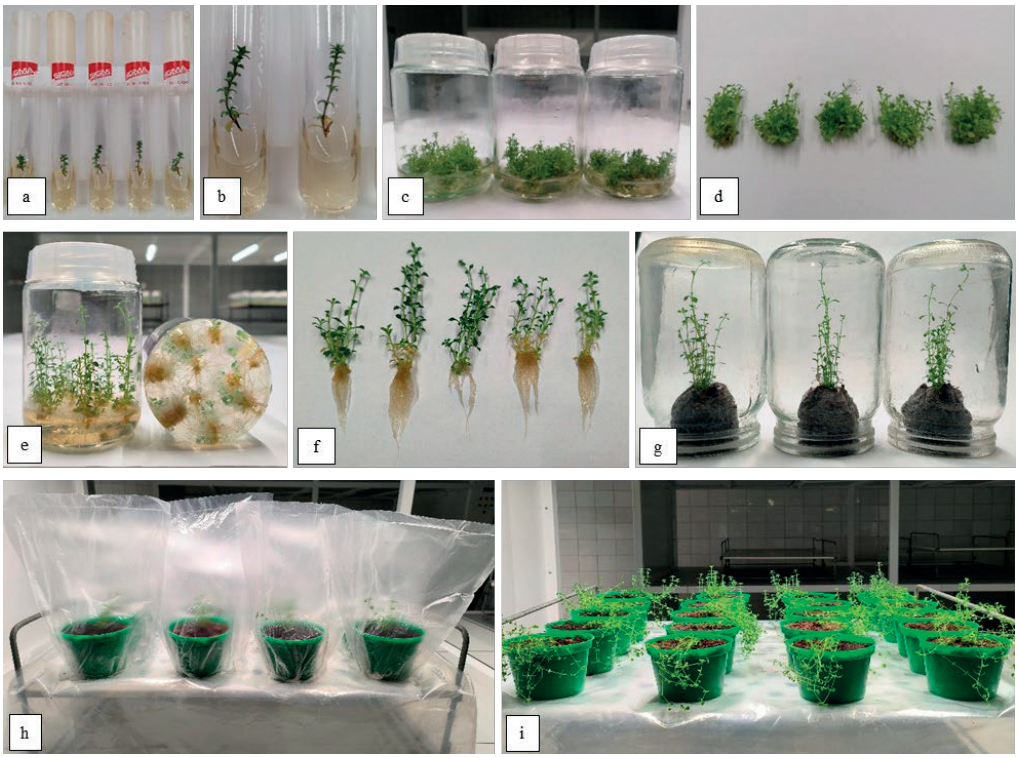


Figure 6. Aspects of *in vitro* culture of *T. x citriodorus*: a, b - explants in the initiation phase on MS medium without PGRs; c, d - shoot propagation on MS medium supplemented with 3 mg/L BAP; e, f - *in vitro* rooted shoots; g - acclimatization of plantlets in Jiffy peat pellets; h - acclimatization of plantlets in a mixture of peat, manure, and perlite (in a ratio of 2:1:1); i - acclimatized plants fortified in pots

Genetic uniformity assessment of *in vitro* regenerated plants

The type and concentration of PGRs, propagation methods, number and duration of subcultures are the main factors influencing the induction of somaclonal variation (Pastelín Solano et al., 2019). According to Kushnir and Sarnatska (2005), in *in vitro* propagation, the number of subcultures should be limited to 10-15. Any extension of these requires additional checks on the genetic stability of the material, as *in vitro* plant tissue culture for a long period of time can induce genetic variability due to prolonged exposure to high concentrations of PGRs (Li et al., 2019). Various molecular techniques have been developed to verify the genetic uniformity of micropropagated plants (Clapa and Hârţa, 2022; Badhepuri et al., 2024; Saptari et al., 2024; Clapa et al., 2025).

In the present study, RAPD analysis was employed to assess the genetic fidelity of *in vitro* propagated *T. x citriodorus* plants.

DNA from the mother plant, *in vitro* propagated plantlets, and acclimatized plants was analyzed for the presence/absence of bands. Scoring of RAPD bands in a binary matrix (1 = present, 0 = absent) revealed 100% similarity among all samples (Bakhtiar et al., 2014).

The concentration and purity of extracted DNA were determined spectrophotometrically. The absorbance ratios (A260/A280) ranged from 1.8 to 2.0, indicating high-purity DNA, free of protein contaminants. The yields were consistent across all samples, and the extracted DNA was found to be of sufficient integrity and concentration, being suitable for subsequent PCR amplification.

Amplification with RAPD primers produced clear and reproducible banding patterns in all ten plants examined. These results are consistent with those reported in other studies conducted on the species *T. persicus* (Bakhtiar et al., 2014) and *T. quinquecostatus* (Kim et al., 2020). Representative profiles are shown in Figure 7.

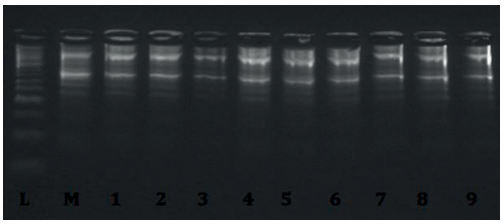


Figure 7. Representative RAPD electrophoretic profiles obtained with primer OPA-09. Lanes 1-10: DNA from mother plant, *in vitro* propagated plantlets (four stages), and acclimatized plants (five individuals). L: DNA ladder (100 bp)

For all primers used, the electrophoretic patterns were similar in the donor plant, four *in vitro* propagated shoots at different stages, and five acclimatized plants. All primers generated a comparable number of bands, ranging from 3 to 8 per primer, with fragment sizes between 200 and 2000 bp. No polymorphic bands were detected in any of the analyzed samples, indicating high genetic stability of the *in vitro* propagated *T. x citriodorus* material. These results confirm that *in vitro* culture did not induce detectable somaclonal variations. Similar results have been reported in other studies conducted on the species *T. persicus* (Bakhtiar et al., 2014) and *T. quinquecostatus* (Kim et al., 2020).

The use of RAPD markers has proven effective for monitoring genetic uniformity, providing a rapid and reliable tool for quality control of tissue culture-derived plants. The total number of bands, their sizes, and reproducibility underline the robustness of the RAPD method for detecting potential variations.

CONCLUSIONS

The use of 6% $CaCl_2O_2$ for the disinfection of explants and the MS medium without PGRs for their inoculation proved effective in inducing *in vitro* regenerative processes in the species *T. x citriodorus*. The highest shoot multiplication rate was obtained on MS medium containing 3 mg/L BAP. The longest shoots were generated in the presence of 0.5 mg/L 2iP and 5 mg/L GA_3. The most effective culture medium for *in vitro* shoot rooting proved to be ½MS medium supplemented with IBA (0.5 or 1 mg/L) or with IBA (0.5 or 1 mg/L) in combination with GA_3 (3 mg/L). *In vitro* regenerated plants were efficiently acclimatized to *ex vitro* conditions. Molecular analyses confirmed the genetic fidelity of micropropagated plants. These results validate the applied micropropagation protocol as a reliable strategy for clonal propagation and germplasm conservation of *T. x citriodorus*.

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THE IMPACT OF THE USE OF GREEN CROPS IN AGRICULTURAL PRODUCTION - ON OVERVIEW

Mădălin RADU, Mihai GÎDEA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihai.gidea@usamv.ro

Abstract

Cover crops are an essential agroecological practice for sustainable agriculture, with multiple beneficial effects on soil, biodiversity and productivity. An integrative analysis of 59 international and national studies shows that they contribute to improving the physical, chemical and biological properties of the soil, reducing nutrient losses and increasing the resilience of agroecosystems. From a physical point of view, green crops increase water infiltration rates by up to 35% and reduce soil bulk density by 6-9%. Chemically, replacing fallow land with green crops reduces nitrate leaching by 45–83%, sometimes equivalent to a reduction of 40-80 kg N ha^{-1} in mineral fertilisation. Biologically, they increase microbial biomass by 20-25% and fungal diversity by 15%, stimulating enzymatic activity and nutrient recycling. A major aspect is carbon sequestration, with average increases of 0.3-0.5 Mg C ha^{-1} year^{-1}, visible especially after 3-5 years of application. Thus, green crops contribute to climate change mitigation and long-term fertility maintenance. In addition, grasses (e.g. rye, oats) are effective for nitrate capture and soil protection, legumes (e.g. clover, vetch) for nitrogen supply, and cruciferous plants (e.g. mustard, radish) for biofumigation and decompaction.The effects on biodiversity and weed control are significant, reducing infestations by 30-45%. In contrast, under optimal conditions, cover crops stabilise yields and reduce costs by decreasing chemical inputs, generating net economic benefits of €60–120/ha. Cost constraints and competition for resources can be overcome by choosing the right species and integrating them into agricultural support policies (e.g. CAP eco-schemes). Their widespread adoption can make a decisive contribution to the transition to more resilient, efficient and environmentally friendly agriculture.

Key words*: cover crop, carbon sequestration, enzymatic activity.*

INTRODUCTION

Modern agriculture faces the dual challenge of ensuring food security and protecting natural resources. *Cover crops* (or *green manure*) have been promoted globally as a solution to improve soil fertility, reduce pollution and increase the resilience of agricultural systems (Silva et al., 2024; Wittwer et al., 2017).

In the US, the area cultivated with cover crops increased from 1.5 million ha in 2005 to over 6 million ha in 2017 (Basche and DeLonge, 2019). In the European Union, they are integrated into CAP schemes, and Romania has begun to encourage their use through eco-schemes and local research, especially on pre-luvisolic soils (Gîdea et al., 2010). Globally, the area cultivated with green crops has grown steadily over the last two decades, estimated at over 15 million ha in the United States and approximately 3 million ha in the European Union (Blesh, 2018; Blanco-Canqui, 2022). However, the adoption rate differs significantly between regions, being higher on organic farms and lower in conventional systems (Blanco-Canqui and Ruis, 2020).

The importance of green crops derives from multiple scientifically documented benefits: carbon sequestration (Blanco-Canqui and Ruis, 2017; Blanco-Canqui et al., 2023), reduction of nitrate leaching (Blanco-Canqui et al., 2015; Brennan and Smith, 2005), improvement of soil physical properties, weed control (Dabney and Delgado, 2001; Fernando et al., 2023) and support for biodiversity (Finney and Kaye, 2017).

However, there are also challenges: competition for resources (water and nitrogen) (Finney et al., 2016), additional costs for farmers (Gîdea et al., 2010) and the risk of negative effects in dry years (Ciontu et al., 2011).

MATERIALS AND METHODS

The paper presents the results obtained as a result of analyzing the specialized literature by accessing electronic databases, including Google Scholar, Science Direct, Scopus and PubMed, of research focused on the benefits of green manure cultivation and on establishing that these can be sustainable crops.
We used the search terms "green manure", "economic importance of green manure" and thus accessed numerous scientific works, as can be observed from the references chapter.

RESULTS AND DISCUSSIONS

Types of green crops and plant combinations used

Green crops can be divided into several functional groups, each with specific advantages. Grasses (e.g. winter rye, oats, wheat, triticale) have a dense and deep root system, making them effective for capturing residual nitrates and protecting against erosion (Kaspar et al., 2012; Snapp and Surapur, 2018).
Advantages Reduce nitrate leaching by 45-710% (Nouri A. et al., 2022; Thapa et al., 2018), protect the soil from erosion and increase aggregate stability (Basche and DeLonge, 2019; Blanco-Canqui and Ruis, 2020), control weeds through shading and mulching (Godar et al., 2025; Nouri et al., 2022).
Disadvantages High C/N ratio → slow decomposition (Blanco-Canqui and Ruis, 2017), May reduce subsequent crop yields in dry years (Kaspar et al., 2012; Wayman et al., 2015).
Legumes (e.g. red clover, field peas, alfalfa, vetch) biologically fix atmospheric nitrogen, providing the equivalent of 40-80 kg N/ha to subsequent crops.
Advantages: Fix atmospheric nitrogen, 40-80 kg N/ha, (Kaye and Quemada, 2017; Tribouillois et al., 2015); improve soil structure and water retention capacity (Finney and Kaye, 2017) contribute to increased microbial biodiversity (Joshi et al., 2023; Thapa et al., 2018).
Disadvantages Requires inoculation with fixing bacteria for maximum efficiency (Blesh, 2018), May compete with the main crop if not finished in time (Kannberg et al., 2024; Wayman et al., 2015).
Cruciferous species (e.g. fodder radish, white mustard) are used for their biofumigant effect and for soil decompaction, as they have deep taproots (Brennan and Smith, 2005; Koudahe et al., 2022). Other species, such as buckwheat, millet or Sudanese sorghum, can be used in diversified systems for organic matter input and weed suppression (Nichols et al., 2020; Ranaldo et al., 2020).
Advantages taproot reduces compaction (Koudahe et al., 2022), biofumigant effect → reduces nematodes and pathogenic fungi (Brennan and Smith, 2005), short cycle → can be introduced between horticultural crops (Murrell et al., 2017).
Disadvantages sensitive to frost, mustard and rapeseed species (Yousefi et al., 2024), can transmit cruciferous diseases if rotations are not respected (Koudahe et al., 2022).
Buckwheat (*Fagopyrum esculentum*) advantages: quickly suppresses weeds and attracts pollinators (Nichols et al., 2020).
Disadvantages: total biomass is lower than that of grasses (Ranaldo et al., 2020).
Phacelia (*Phacelia tanacetifolia*) advantages: melliferous, supports biodiversity and pollination (Wittwer et al., 2017).
Disadvantages: does not fix nitrogen, requires dense sowing for complete coverage (Yousefi et al., 2024).
Sudanese sorghum/pearl millet advantages: produces high biomass, drought tolerant (Ranaldo et al., 2020). Disadvantages: sensitive to cold, can compete intensely for water (Kannberg et al., 2024).
In practice, mixtures of species that combine the advantages of different groups are frequently used. Finney et al. (2016) and Ranaldo et al. (2020) showed that mixtures of grasses and legumes provide both nitrate capture and biological nitrogen supply, simultaneously increasing total biomass and the suppressive effect on weeds.
In Europe, adapted mixtures (e.g. rye + vetch, oats + peas, triticale + clover) have demonstrated superior performance to monocultures, with higher productivity and increased resilience to variable climatic conditions (Wittwer et al., 2017).

In Romania, long-term experiments have confirmed that rotations with complex mixtures can ensure stable yields and maintain the fertility of pre-luvisolic soils (Ciontu et al., 2011; Gîdea et al., 2010)

Influence on soil physical properties

Green crops contribute significantly to improving soil structure and stability.

Basche and DeLonge (2019) showed that infiltration rates increase by 35% in systems with cover crops.

Blanco-Canqui and Ruis (2020) confirm a 6-9% reduction in apparent soil density and a 15-20% increase in aggregate stability.

Finney and Kaye (2017) demonstrated that the functional diversity of cover crop mixtures increases root biomass by 18%, improving soil structure.

In Romania, wheat and maize rotations on pre-luvisolic soils reduced compaction and improved water retention (Ciontu et al., 2011; Gîdea et al., 2015).

Dabney et al. (2001) showed that soil losses through erosion are reduced by 40-60% on sloping land, confirming the role of cover crops in soil conservation.

In another study, Finney and Kaye (2016) showed that the functional diversity of cover crop mixtures contributes to better soil structure, with an 18% increase in root biomass and a positive effect on microaggregate stability.

Blanco-Canqui (2022) highlighted the role of deep-rooted species (e.g. rye, fodder radish) in reducing compaction and improving porosity.

The effects on soil erosion are also notable. Dabney et al. (2001) showed that the use of cover crops reduces soil loss through erosion by 40-60%, especially on sloping land. This effect derives both from the coverage of the soil with plant debris and from the ability of the roots to stabilise aggregates and reduce surface runoff.

Influence on soil chemical properties

Tonitto et al. (2006), analysing 37 studies, showed that replacing fallow land with cover crops improves the nitrogen cycle and reduces losses. Thapa et al. (2018) reported average reductions in nitrate leaching of 70%, while Nouri et al. (2022) confirm maximum values of 83%. Kaspar et al. (2012) observed decreases in nitrates in drainage water of between 25-60%.One of the main benefits of green crops is their ability to contribute to the storage of organic carbon in the soil.

The meta-analysis by Poeplau and Don (2015), which included over 100 experiments, showed that the use of cover crops increases carbon stocks by an average of 0.32 ± 0.08 Mg C ha^{-1} $year^{-1}$.

The effect is more pronounced in degraded soils and temperate climates, where carbon accumulation can exceed 0.5 Mg C ha^{-1} $year^{-1}$ (Blanco-Canqui et al., 2023).

Another global study (Gîdea et al., 2015) showed that integrating green crops into rotations contributes to an increase in active soil carbon content of 12-20% after 5 years of continuous application. This is essential for achieving the climate goals set out in the Paris Agreement and for the resilience of agroecosystems to climate change.

Reducing nitrate leaching

A major, extensively documented benefit is the reduction of nitrate losses from the soil through leaching. Thapa et al. (2018) conducted a global meta-analysis of 131 studies and showed that the use of cover crops reduces nitrate leaching by an average of 70%, with variations between 45% and 92%, depending on species and management.

Nouri et al. (2022) confirmed these results, emphasising that efficiency is highest in temperate climates, where the reduction reaches up to 83%.

One of the most important benefits of green crops is their ability to increase soil organic carbon stocks, thereby contributing to climate change mitigation and long-term fertility improvement.

Global meta-analyses show that the use of cover crops leads to an average increase of 0.3-0.5 Mg C ha^{-1} $year^{-1}$. Poeplau and Don (2015), analysing over 100 experiments, confirmed that the largest increases occur in systems with diverse rotations and moderate residue inputs. Jian et al. (2020) showed that the effect is cumulative: carbon gains become visible after 3-5 years and continue to increase in long-term experiments.

Blanco-Canqui (2022) emphasises that perennial grasses and legume + grass mixtures have the highest sequestration potential because they provide both a large amount of biomass and residues with variable C/N ratios, which promotes balanced mineralisation. Tiemann et al. (2015) showed that species diversity stimulates microbial activity, which accelerates the stabilisation of organic matter. Ruis and Blanco-Canqui (2017) highlighted that the benefits are more pronounced in degraded or eroded soils, where additional inputs of organic matter can quickly restore carbon balance.

Wittwer et al. (2017), in a European synthesis, confirm that in temperate regions, cover crops can compensate for up to 25-30% of carbon losses caused by monoculture and intensive farming.

In Romania, studies on pre-luvisols and southern chernozems (Ciontu et al., 2011; Gîdea et al., 2008; 2010; 2012; 2015) have shown that rotations with legumes and grasses increase humus content by 0.2-0.3% after 5-7 years of application. These data confirm that the widespread adoption of green crops could be an effective strategy for increasing the resilience of agroecosystems to climate change.

Waring et al. (2020) showed that introducing rye as a green manure crop reduces nitrate concentrations in groundwater drainage by 25-60%, while modelling studies by Malone et al. (2014) and Gupta et al. (2022) suggest that nitrate losses could be reduced by more than 50 kg N ha^{-1} $year^{-1}$ in artificially drained systems.

With regard to phosphorus, Koudahe et al. (2022) showed that plant residues reduce losses through surface runoff.

In experiments in Romania, rotations with peas and wheat led to better mineral fertiliser efficiency and increased humus content (Gîdea et al., 2008; 2010; 2012; 2015).

Influence on soil biological properties

Soil biological activity is significantly stimulated by green crops (Zhou et al., 2021).

Tiemann et al. (2015) reported increases in microbial biomass by 22% and fungal diversity by 15%.

Tribouillois et al. (2015) showed that legumes stimulate nitrogen-fixing bacteria and reduce pathogens.

Jian et al. (2020) confirmed that cover crops intensify the carbon cycle by stimulating the microbiota.

In Romania, complex rotations on pre-luvisolic soils have led to significant increases in microbial biomass and biological fertility (Ciontu et al., 2011; Gîdea et al., 2015).

Influence on soil enzyme activity

Soil enzymes are indicators of quality and fertility (Zală, 2015).

Jian et al. (2020) showed that cover crops increase β-glucosidase and urease activity.

He et al. (2025) confirmed that legumes enhance phosphatase activity, increasing phosphorus availability.

In experiments in Romania, Burcea et al. (2007) demonstrated that reduced tillage and crop rotations increase catalase activity and reduce weed infestation.

Influence on biodiversity and weed spectrum

Cover crops reduce weed pressure through shading and mulching effects.

Nichols et al. (2020), based on 67 studies, reported an average reduction of 33% in infestation.

Osipitan et al. (2019) confirm the effectiveness of rye and oats.

Godar et al. (2025) showed that mixtures reduce infestation by 45%, compared to 28% in monocultures.

In Romania, Gîdea et al. (2010; 2012; 2015) showed that rotations and integrated technologies significantly reduce weed pressure on maize and sunflowers.

Influence on pathogens and pests

Murrell et al. (2017) demonstrated that delaying the termination of cover crops can increase the incidence of root diseases in maize by 10-15%, but proper management prevents these effects.

Brennan and Smith (2005) showed that mustard and radish have a biofumigant effect, reducing soil pathogen density by 20-40%.

In Romania, Gîdea et al. (2008) highlighted the reduction in *Pyrenophora tritici-repentis* attack on the Flamura 85 variety in green crop rotations.

Influence on plant productivity, growth and development

The impact of cover crops on yields is variable, but in the long term tends to be positive or neutral.

Tonitto et al. (2006), in a meta-analysis of 37 studies, showed that cover crops stabilise yields and reduce nitrogen losses.

Peng et al. (2024) confirm, through a global synthesis, that average yields do not decrease and interannual variability is reduced.

Silva et al. (2024) reported in the US Midwest that soybeans sown after rye do not lose yield, but even gain modestly (+2–4%).

In contrast, Wayman et al. (2015) and Kannberg et al. (2024) showed that excessive delay in termination can generate competition for water and nitrogen, leading to 5-15% decreases in maize.

Gîdea et al. (2010; 2012; 2015) showed that green crop rotations increase wheat and maize yields by 8-12% in normal years, but can reduce production by 5-10% in dry years.

Influence on rotations

Cover crops improve the efficiency of rotations.

Kaye and Quemada (2017) showed that legumes can provide the equivalent of 40-80 kg N ha^{-1} through biological fixation.

Blesh (2018) and Finney et al. (2017) confirm that legume-grass mixtures combine the advantages of nitrogen fixation with residual nitrate capture.

Gîdea et al. (2010; 2012; 2015) showed that complex rotations reduce weed infestation and increase the efficiency of mineral fertilisers.

Environmental impact

Thapa et al. (2018) and Ritz et al. (2017) showed that nitrogen losses are reduced by 20-70% through the use of cover crops.

Nouri et al. (2022) confirm maximum reductions of 83%.

Waring et al. (2020) observed decreases in nitrate concentrations in drainage of up to 62%.

In terms of gas emissions, Jian et al. (2020) showed that N_2O decreases by 10-20% when cover crops are introduced into agricultural systems.

In Romania, Gîdea et al. (2008; 2010) confirm that crop rotations reduce nitrate pollution and increase biodiversity.

Differentiated influence on crops

Maize: rye reduces nitrate leaching by 60-70% and provides 30-50 kg N/ha (Kaspar et al., 2012).

In Romania, crop rotations increase yields by 5-12% (Ciontu et al., 2011).

Soybeans: benefit from the suppressive effects on weeds, without yield losses (Silva et al., 2024).

Wheat: rotations with legumes provide the equivalent of 40-80 kg N/ha (Kaye and Quemada, 2017); Gîdea et al. (2008) showed a reduction in foliar diseases.

Vegetables: mustard reduces pathogen density by 40% and nitrogen losses by 35% (Brennan and Smith, 2005).

Sunflower: rotations reduce infestation and increase production stability (Gîdea et al., 2008).

For orchards and vineyards → clover, phacelia, rye (biodiversity + fertility).

For greenhouses and field vegetables → fodder radish, mustard (biofumigation), vetch + oats (nitrogen + mulch).

For intensive rotations → grass + legume mixtures (nutrient balance and weed suppression).

Competition for resources

Wayman et al. (2015) and Kannberg et al. (2024) showed that in dry years, cover crops can reduce maize yields through competition for water and nitrogen.

Profitability and economic efficiency

Ranaldo et al. (2020) showed that in Mediterranean systems, net economic benefits can reach €60-120/ha through reduced herbicide and fertiliser use.

Schipanski et al. (2014) calculated initial costs of 40-70 USD/ha, but medium-term savings through reduced inputs by increasing economic efficiency, stabilising production and reducing pesticide costs.

Limiting factors

1. Water resources - competition in arid areas (Kannberg, et al., 2024; Wayman et al., 2015).
2. Costs - additional, USD 40-70/ha (Silva et al., 2024).
3. Management - choice of species and timing of termination are critical (Murrell et al., 2017).
4. Slow benefits - appear after 3-5 years (Blanco-Canqui and Ruis, 2020).
5. Socio-economic barriers - small farms find it harder to adopt (Schipanski et al., 2014).

Advantages

Biological nitrogen fixation: 40-80 kg N/ha (Kaye and Quemada, 2017; Tribouillois et al., 2015).
Reduction of nitrate leaching: 45-83% (Nouri et al., 2022; Thapa et al., 2018).
Carbon sequestration (0.3-0.5 Mg C/ha/year) (Poeplau and Don, 2015; Jian et al., 2020).
Improvement of soil physical properties (Basche and DeLonge, 2019; Blanco-Canqui and Ruis, 2020).
Weed control: 30-45% (Godar et al., 2025; Nichols et al., 2020).
Increased biodiversity and microbiota (Tiemann et al., 2015; Yousefi et al., 2024).
Economic benefits: additional profit 60-120 €/ha (Ranaldo et al., 2020).

Disadvantages

Yield losses in dry years: –10 ...–15% (Kannberg et al., 2024; Wayman et al., 2015).
Additional costs (Schipanski et al., 2014).
Requires complex management (Murrell et al., 2017).
Benefits visible after several years (Blanco-Canqui and Ruis, 2020).

Socio-economic integration

In Europe, cover crops are integrated into CAP and eco-schemes (Wittwer et al., 2017).
In the US, the area has grown exponentially over the last 20 years (Basche and DeLonge, 2019).
In Romania, crop rotations and green crops are vital for adapting to climate change and protecting soils (Gîdea et al., 2008; 2010; 2012; 2015; 2016).
Adoption depends on financial support, technical advice and farmer networks.

Potential for use

He et al. (2025) showed that global adoption of cover crops could reduce GHG emissions from agriculture by 5-10% by 2050.
Jian et al. (2020) confirm that the long-term effects on carbon are cumulative.
In Romania, pre-luvisols and southern chernozems are the most promising for the expansion of cover crops (Gîdea et al., 2008; 2012; 2015).

CONCLUSIONS

The analysis showed that:
Cover crops increase the amount of carbon sequestered in the soil by 0.3-0.5 Mg C/ha/year (Poeplau and Don, 2015; Jian et al., 2020).
They reduce nitrate leaching by 45-83% (Nouri et al., 2022; Thapa et al., 2018).
They improve soil structure and microbiology (Blanco-Canqui et al., 2023; Tiemann et al., 2015).
They reduce weed infestation by 30-45% (Godar et al., 2025; Nichols et al., 2020).
They support biodiversity and reduce GHG emissions (He et al., 2025; Wittwer et al., 2017).
They stabilise yields of main crops (Peng et al., 2024; Tonitto et al., 2006).
However, there are limitations related to costs, competition for resources and management complexity.

RECOMMENDATIONS

1. Choose species according to soil and climate conditions: rye/oats for nitrate capture, legumes for nitrogen supply.
2. Integration of cover crops into 1-2-year rotations to reduce fertilisers by 40-80 kg N/ha.
3. Extension of payments for ecosystem services and financial support through the CAP.
4. Long-term research (& gt; 10 years) to quantify cumulative effects.
5. Use of digital technologies to optimise management.
6. Training programmes and farmer networks for knowledge transfer.
7. Adaptation to climate change: in dry areas → drought-resistant species, in wet areas → species to reduce leaching and erosion.

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DISCUSSIONS ON SOME ALIEN SPECIES FROM THE FLORA OF OLTENIA (ROMANIA)

Daniel RĂDUȚOIU

University of Craiova, 13 A.I. Cuza Street, Craiova, Romania

Corresponding author email: radutoiudaniel@yahoo.com

Abstract

Alien plants from Romania have drawn the attention of researchers since the beginning of this millennium. A first attempt to create an inventory of alien species in the flora of Romania was made about 20 years ago. In less than 10 years, the number of such plants included on the list has increased by about 35%. Annually, contributions are made to the spread of these taxa or new taxa are mentioned for the adventitious flora of Romania. In this paper, the author discusses certain alien taxa that develop on areas located in southern Romania (especially in Oltenia). The following species are analyzed: Echinocystis lobata, Amaranthus palmeri, Azolla filiculoides, Vallisneria spiralis, Asclepias syriaca, Prunus cerasifera, Oenothera glazioviana, Abutilon theophrasti, Datura wrightii, Symphyotrichum lanceolatum, Eleusine indica. Voucher specimens collected in the field are deposited in the Herbarium of the University of Craiova.

Key words*: alien species, comments, new records, Oltenia, Romania.*

INTRODUCTION

Studies related to alien plants/species have been known for more than half a century at the European level and beyond. In Romania, these studies become more frequent after the year 2000. Until that time, the adventive species were included in various floristic overviews and association tables (Țopa, 1962; 1972; Buia & Păun, 1964; Dihoru et al., 1972; 1973; Dobrescu et al., 1962; Morariu, 1963; 1966; Ștefureac et al., 1971; Roman & Roman, 1961; Roman & Babaca, 1966; Roman, 1974; Popescu, 1974; Matacă, 2003; Răduțoiu, 2004; Costache 2005), without paying special attention to them.

The numerous scientific works that have been elaborated since 2005 until the present contain information about this category of plants at the national level (Anastasiu & Negrean, 2005; 2007; Oprea et al., 2005; Georgescu & Luchian, 2023; Sârbu & Oprea, 2011; Anastasiu et al., 2024), as well as from the Oltenia region (Costache & Răduțoiu, 2005; Oprea et al., 2005; Răduțoiu, 2006; 2009; Răduțoiu & Costache, 2008; Răduțoiu & Stan, 2013; Răduțoiu & Popescu, 2020; Răduțoiu & Ștefănescu, 2016; 2022; Răduțoiu & Niculescu, 2023; Răduțoiu et al., 2010; 2023a; 2023b; Răduțoiu, 2011; 2024; Răduțoiu & Băloniu, 2024).

The first attempt to draw up an outline of the adventive species in Romania was made in 2005 (Anastasiu & Negrean, 2005). The data gathered over time supplemented this inventory, so that in 2011 almost 1/3 of the information existing at that time was added, as 671 adventive taxa were inventoried (Sârbu & Oprea, 2011), while their current number reaches 740 taxa (Kalusová V. et al., 2024; Anastasiu et al., 2024).

Some of these species have become invasive, representing a real danger to natural and anthropogenic habitats (Pimentel et al., 2000; McNeely, 2001; Wittenberg & Cock, 2001).

Year by year, the effects of climate changes worsen and affect plants worldwide, shifting plant communities, resulting in changes in flora biodiversity and disturbing their life cycles and function (Sărățeanu et al., 2023). In this context, there are required certain measures for restoring ecosystems as efficient tools for reducing climate related risks (Acatrinei et al., 2024; Durău et al., 2021; Sălceanu et al., 2023; Velea et al., 2021).

The present paper supplies some information related to certain adventive plant species that develop on areas located in Oltenia. The research provides details regarding their phenology and distribution in this part of the country.

MATERIALS AND METHODS

The species mentioned here were identified during numerous fieldtrips conducted in different areas of the Oltenia region (Romania) (Figure 1). GPS coordinates were recorded and photographs were taken for the identified species, some of these recordings being part of the present work. The analysis of the characters required for correct identifications was conducted in the specialized laboratory of the University of Craiova and it was based on the field literature (Tutin et al., 1964-1980; 1993; Ciocârlan, 2009; Sârbu et al., 2013), as well as on the herborized material. The collected specimens are included in the Herbarium of the University of Craiova (CRA). Since certain invasive species develop on increasingly larger surfaces, for the near future it is recommended to use the arial photography technology enabled by drones, in order to have overview images of the surfaces occupied by these plants (Călina et al., 2020).

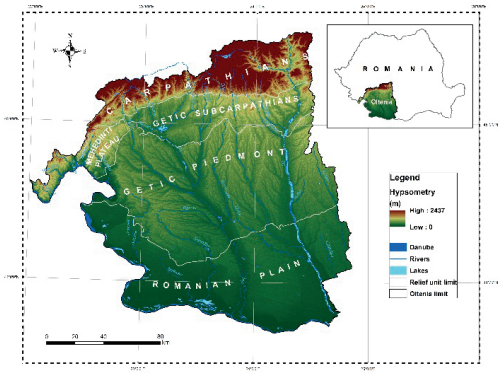


Figure 1. Map of Oltenia and location of the region at the national level (Source: GIS processing after Topographical Map, 1:25,000)

The terminology used in the present paper is consistent with that in the specialized literature that addresses the status of this category of plants (Pyšek et al., 2004).

RESULTS AND DISCUSSIONS

The research conducted during recent years in the Oltenia region (Romania) has also focused on adventive plants, among others, because the field observations made over time revealed that many of them settle in new natural or anthropized territories, where they induce significant changes in the floristic composition of those areas. Certain species for which obvious changes in phenology and distribution have been observed are presented below.

Abutilon theophrasti (*Malvaceae*)

Opinions are divided with respect to the origin of this species (Sîrbu & Oprea, 2011), but whatever that may be, one thing is certain: the species is currently found all over the world, except for Central and South America (Stegink & Spencer, 1988; Warwick & Black, 1988; Pheloung et al., 1999; Ali & Qaiser, 2001; Ciocârlan et al., 2004, etc.). In Romania, it is mentioned as a weed in agricultural crops from almost all regions of the country. In Oltenia, it prefers corn crops, but it has also been observed in sorghum crops, on the territory of Olt County. In the specialized literature, it is mentioned in the plain areas and piedmont hills, which can be explained by the fact that it is a heliophilous and thermophile species; however, the climate changes during recent years have enabled this taxon to advance at the level of the Subcarpathian depression in Oltenia as well (e.g., Pauşeşti-Otăsău settlement in Vâlcea County). Within the settlements situated along the Danube River (e.g., Orlea - Olt County, Bechet and Rastu Vechi - Dolj County) this plant forms abundant populations, while in the Subcarpathian depression its presence takes the form of solitary individuals, in corn crops and vegetable gardens. If the climatic conditions continue the same trend, it is possible that the future representation of the species will be much higher in this area as well. At the level of the Subcarpathian depression, many of the identified specimens do not form flowers and they only develop a weak vegetative apparatus.

New locations: Negoiești, Melinești, Rastu Vechi, Vela, Desnățui, Podari, Palilula, Cetate, Radovan, Coșoveni, Sadova, Valea Stanciului, Gângiova settlements (Dolj County), Mateești and Crețeni settlements (Vâlcea County), Urdari settlement (Gorj County).

Amaranthus palmeri (*Amaranthaceae*)

The species is native to Mexico and to the United States (Brenan, 1961). In Europe, it has been known since the beginning of the 20th century, from the Czech Republic (around 1908) (Pyšek et al., 2004). Subsequent research highlighted not only the presence of this plant in

other European states (Aellen, 1959; Brenan, 1961; Sauer, 1967; Frey, 1974; Carretero, 1990; Mosyakin, 1995; Raus & Raabe, 2006; in Greuter & Raus, 2006; Dimopoulos et al., 2013), but also its success in the competition with other weeds that coexist with it (Sîrbu & Oprea, 2011).

In Romania, it is mentioned for the first time by Costea (1996; 1997) from Constanța and Bucharest railway station. The first herbarium material with this taxon dates from 1981 and was collected by G. Negrean (Sîrbu & Oprea, 2011). Subsequently, it was found in other places in the country (Oprea et al., 2021).

Although this taxon grows in both ruderal and segetal anthropogenic habitats, in Oltenia it is present only near the railways, in sandy, well-aired places, within the plain region. If the evolution of the climatic conditions in southern Romania is taken into account, we can state that the area of this species will increase considerably in the near future.

It is the first mention of this taxon in Olt County - at the railway stations of Drăgănești-Olt (44.153835 °N; 24.546436°E) and Caracal (44.120792°N; 24.364594°E) towns, among the railway tracks (Figure 2) and the second mention in Dolj County - from ruderal places located on the outskirts of Voita settlement (Brabova commune) (44.364750°N; 23.386570°E).



Figure 2. *Amaranthus palmeri* from the Drăgănești-Olt railway station

Asclepias syriaca (*Asclepiadaceae*)

It is a taxon of interest for the EU and its development area within the Oltenia region has recently expanded. The species originates in eastern Canada and in the area extending from the eastern to the central United States of America (Frankton & Mulligan, 1974), from where it also reached Europe (Bagi, 2008; in Botta-Dukát & Balogh, 2008).

It was initially introduced only as cultivated plant, but subsequently become subspontaneous in different regions of Europe (Bagi, 2008; in Botta-Dukát & Balogh, 2008), including Romania, thus being naturalized in numerous European states. The first chorological data concerning the presence of this species in Oltenia are from the Danube Floodplain (Răduțoiu & Stan, 2013). Subsequently, it was found that this taxon began to expand its growing area within the Oltenia Plain, the Getic Piedmont, as well as in the Subcarpathian Depression (Răduțoiu & Băloniu, 2021).

In recent years, it has also been identified in other counties of Oltenia: Vâlcea (Frâncești settlement), Gorj (Cojani - roadside, corn crop and *Agrostis capillaris* meadows (44.992908°N; 23.531448°E), Jupânești), and Dolj (Braloștița, Preajba, Maglavit, Murgași, Teasc, Poiana Mare, and Zăval settlements). Almost all identified specimens abundantly bloom and fructify over a long period of time (from May until October).

Azolla filiculoides (*Azollaceae*)

With respect to the origin of this taxon, the specialized literature mentions it as native to the North, Central and South America, from where it was also introduced to Europe (West, 1953 in Hussner, 2006). Its first mention is from France. Subsequently, it is found in the flora of numerous European states. In Romania, it has been known since the beginning of the 20th century (Prodan, 1939), from the Danube Delta and from the marshes around Bucharest (the Cernica Monastery). To date, it is mentioned from ten counties (Sîrbu & Oprea, 2011; Popescu et al., 2001; Răduțoiu & Boruz, 2001), two of them being located in Oltenia (Dolj and Mehedinți Counties).

Although this plant is included in the Red Book of vascular plants in Romania as a vulnerable species (Dihoru & Negrean, 2009), at the European level, its status is that of an invasive species. The increasing number of places where it has been identified within the counties already mentioned in the field literature and the significant expansion in other Romanian counties (those located in Oltenia implicitly) represent evidences that enable us to state that

this taxon will continue to expand its development area in the near future.

New locations: in the vicinity of the Danube bend, next to Ciupercenii Noi settlement; in a canal within the Danube Floodplain, next to the village of Desa (Dolj County); in the Olt River floodplain, next to Gostavățu settlement (Olt County).

Datura wrightii Regel (*Solanaceae*)

This taxon is native to the southwestern United States of America and Mexico (Verloove, 2008). At the European level, this species is known as an ornamental plant, but in recent years it has been mentioned as having fallen out of cultivation in a growing number of countries, such as Spain (Verloove, 2008), Austria (Essl & Rabitsch, 2002), France, including the island of Corsica (Lambinon, 2006, quoted by Verloove, 2008), Hungary (Király et al., 2009), and Romania (Sîrbu & Oprea, 2011; Oprea et al., 2015; Camen-Comănescu & Mihai, 2022; Niculescu, 2022; Şuşnia, 2022; Camen-Comănescu et al., 2023; Nagodă et al., 2023). It is also mentioned from Tunisia and Australia (https://powo.science.kew.org).

In Oltenia, it is mentioned from the counties of Vâlcea (Râmnicu Vâlcea, Băile Govora, Bălcești, and Nicolae Bălcescu) and Dolj (between Segarcea and Giurgita, Craiova, Cârna and in Măceșu de Sus) (Niculescu, 2022).

It was also identified in Poiana Mare, Sârsca, Sopot, and Potmelțu settlements within Dolj County, as well as in Spineni and Drăgăneşti settlements within Olt County.

Echinocystis lobata (Michx.) Torr. & A. Gray (*Sicyos lobatus* Michx.) (*Cucurbitaceae*)

It is an adventive plant that originates in North America and prefers river floodplain habitats (Foster & Duke, 1990). In Oltenia it is found in the Danube Floodplain (where it forms impenetrable thickets, such as those located near the Port of Rast), as well as in the inland river floodplains (e.g., those of the Jiu and the Olt).

At the beginning of this century, the species in question was mentioned in the Romanian specialized field guides as a rare plant. In recent years, this taxon has undergone a luxuriant development, especially in the lower part of the river floodplains and in the Danube Floodplain. The spread over larger areas is due to the climatic conditions in this part of the country, characterized by higher temperatures over a longer period and by stronger and more frequent winds. These changes allow the plant to form more flowers, fruits and seeds that are easily dispersed by wind. Unless urgent measures are taken, in the near future, this taxon will fully contribute to the deterioration of the Natura 2000 habitat: 92A0 *Salix alba* and *Populus alba* galleries.

New locations: The settlements of Secui (Figure 3), Bădoși, and Bratovoiești (Dolj County).



Figure 3. Fruit-bearing specimen from the Jiu floodplain, near Secui settlement

Eleusine indica (*Poaceae*)

It represents a grass and the data regarding its origin are different. Some authors specify that this plant originates in the tropical and subtropical part of Asia (Ciocârlan, 2009; Oprea, 2005), while others place its origin in Africa (Hildebrand, 2008). It is currently found on all continents, especially in tropical and subtropical areas (Hitchcock, 1950; Britton & Brown, 1970; Jürgens, 1977; Salimath et al., 1995; Clayton et al., 2006).

In Romania, it was reported as a subspontaneous plant from Iași County (Răvăruț & Mititelu, 1960). Subsequently, new information appeared from the north-western Romania (Negrean & Karácsonyi, 1984), Dobrogea region (Costea, 1996), and Muntenia region (Negrean & Constantin, 1999; Oprea et al., 2004).

The first mention of this taxon in the flora of Oltenia is from Dolj County (Răduțoiu & Stan, 2013).

In recent years, an increasing spread has been noticed in this part of Romania. It is important to mention that the identified populations are sparse and cover small surfaces, located near more humid areas.

It was identified in settlements situated in Vâlcea County (Păușești-Otăsău, Pietrari, and

Buneşti), as well as in Olt County (Gostavăţu settlement).

Oenothera glazioviana (*Onagraceae*)

The taxon is currently characterised by a wide global distribution, except for Antarctica, being found either as a cultivated plant for ornamental purposes, or as a subspontaneous plant (Ali & Qaiser, 2001; Weakley, 2007). It is native to North America, from where it has spread almost all over the Earth. In Romania, it is reported from several counties (Morariu in Săvulescu, 1957; Oprea & Sîrbu, 2006; Sîrbu & Oprea, 2008; 2010). In the places mentioned within the specialized literature, the species is present under the form of isolated specimens, this aspect being also observed by the author in the locations where it was found for the present study. In Oltenia, the plant in question prefers sandy, open, and well-aired places, roadsides, ruderal areas, especially on the banks of rivers where the local inhabitants throw vegetable remains from the specimens grown in their own households. The species does not tolerate competition, thus preferring pioneer places where it can develop its entire biological cycle. In phytocoenosis with uniform physiognomy and large coverage, this plant develops only the vegetative apparatus.

In Oltenia, it is the most widespread species of the genus Oenothera. Basically, this taxon can be identified in all settlements.

Examples: the cemetery on the outskirts of Covei settlement; ruderal places, sidewalk, near Calafat municipality Police; in an abandoned vineyard on the outskirts of Poiana Mare settlement; on the side of a road in Ciutura settlement; in xeric meadows with abundant *Cynodon dactylon*, which are located in Salcia, Maglavit, and Georocul Mare settlements (Dolj County), in ruderal places from Păuşeşti-Otăsău settlement (Vâlcea County).

Prunus cerasifera (*Rosaceae*)

This plant has taken full advantage of all the opportunities it has been given in terms of spreading and occupying new territories. According to some authors, this taxon is considered to be indigenous only in South-Eastern Europe (Sîrbu & Oprea, 2011), while on the rest of the continent it is cultivated and locally naturalized (Austria, Great Britain, Denmark, France, Germany, Hungary, Italy, and Romania). About a quarter of a century ago, this plant was subspontaneous in Oltenia, being found under the form of isolated specimens, while its current populations are abundant, especially in the plain region of Oltenia. This can be explained either by the frequent reproduction by generative means, which is rather often noticed in the field, and by the appearance of root suckers, or by the resistance of this plant to frost and drought (Sonea, 1957), which are quite common phenomena in the southern part of Oltenia.

In order to understand the representation of this taxon in a certain region of Oltenia, it is enough to notice the vernal aspect, when this plant is in bloom.

The current climatic conditions in this part of Romania, associated with the abandonment of certain ruderal and segetal lands, will enable this plant to increasingly invade the anthropized and semi-natural habitats in the near future. The fruit-growers represent the only researchers who can benefit from the presence of large populations of *Prunus cerasifera*, as they use cherry plum specimens as rootstocks for plum, peach and apricot trees (Buia in Săvulescu, 1956; Sonea, 1957).

The fight against this species is difficult in Oltenia, because of the multiple agents that led to its spread: humans, but also animals, either domestic (especially sheep and goats) or wild (deer, wild boar, and various bird species).

New locations: Calafat Customs, Breasta, the outskirts of Leu settlement, Castranova, Bordei train stop located near Craiova, Filiaşi and Segarcea railway stations, the settlements of Prunet, Tatomireşti, Obedin, Scăeşti, Sfârcea, Mischi, Goieşti, Negoieşti, Melineşti, Drănic, Ghidici, Băileşti, Vela, Pietroaia, the edge of Urdinița cemetery, between Malu Mare and Ghindeni, between Bucovăț and Lazu, Terpezița, Brabova, Răchita de Sus, Carpen, Lăcrița, Vârvor, Zănoaga, Bulzeşti, Coşoveni, Verbicioara, Verbița, Podari, Leamna de Jos, Leamna de Sus, Motoci, Murgaşi, Picăturile, Dobreşti, Drănic, Maglavit, Pieleşti, Bratovoieşti, Criva, Dobromira, Ciutura, Țuglui, Crovna, Fratoștița, Râșnic, Cârcea, Almăj, Rovine, Teslui, Cornița, Ștefănel, Gogoşu, Robăneşti, Drgăgoteşti, Lipovu, Roşieni, Țiu, Întorsura, Goicea, Drăgoaia, Lişteava, Gângiova, Ostroveni, Piscu Vechi, Poiana Mare, Plenița, Moțăței, Seaca de Câmp, Valea

Stanciului, Tâmburești, Comoșteni, between Daneți and Dobrotești (Dolj County), the Port of Corabia, Orlea, Caracal, Slatina railway station, the outskirts of Balș town and those of Brănet, Brâncoveni, Bobicești, Piatra Olt, Oltișoru, and Bârza settlements (Olt County), Bumbești-Pițic, on the banks of the Galbenu river, Novaci, (Gorj County), Burila Mare settlement (Mehedinți County), Drăgășani, Păușești-Otăsău, Pesceana, Tomșani, Slătioara, Gorunești, Stroești, Păsculești, Fârtățești, Giulești, Crețeni, Gușoeni, Glăvile, Amărăști, and Călimănești-Căciulata (Vâlcea County).

Symphyotrichum lanceolatum (*Asteraceae*)
It is known as a native species from North America (Britton & Brown, 1970) and it was subsequently introduced into Europe as a plant cultivated for ornamental purposes and then naturalized (Hoffmann 1996, Wittenberg, 2005, Fehér, 2008).
The first report of this taxon in Oltenia is from Mehedinți County, namely from the town of Orșova (Morariu et al., 1969). More recently, it has been also indicated in the Habitat 3130 - Oligotrophic to mesotrophic standing waters with vegetation of the Littorelletea uniflorae and/or Isoëto-Nanojuncetea (CLAS. PAL.: 22.12 x (22.31 and 22.32), from the island of Copanița (Dolj County) (Niculescu et al., 2023), from phytocoenosis belonging to *Cyperetum flavescenti* Koch ex. Aichinger 1933, *Juncetum bufonii* Felfoldi 1942 associations.
New locations: The Danube Floodplain, near the Ports of Rast and Bechet, in places with sparse vegetation, on the edges of wet scrub areas and in ditches located near roads in poplar forests.

Vallisneria spiralis (*Hydrocharitaceae*)
This species holds a controversial status in the flora of Romania. The *Flora Europaea* (Dandy, in Tutin et al., 1980) specifies that this taxon is native to Romania, whereas most of the Romanian specialized literature mentions it as an adventive plant (Beldie, 1979; Ciocârlan, 2000; 2009; Oprea, 2005; Chifu et al., 2006; Sârbu et al., 2013).
From Oltenia, it is known from a small number of settlements; thus, it is mentioned from Rast and Bistreț, without field identification or herbarium material (Popescu et al., 2001), Strâmba Brook (Ro: *Gârla Strâmbă*) from Bistrețu village - Mehedinți County (Țopa in Săvulescu, 1966, Oprea, 2005), the Iron Gates I and II (Liţescu et al., 2003), the Preajba-Făcăi Lacustrine Complex (Răduțoiu & Răduțoiu, 2024, Răduțoiu, 2024).
The development of almost monodominant populations of this species in an increasing number of areas located in Oltenia can also be triggered by the augmented temperatures in this part of Romania, as it is known that this taxon optimally develops at temperatures higher than 20°C (Hutorowicz, 2006).
New locations: in a canal located within the Danube Floodplain, in the area of Desa settlement.

CONCLUSIONS

The analysis of the species tackled in this paper reveals that most of them prefer regions with higher temperatures, these climatic conditions being found in the southern part of Romania. The combination of climate changes in recent years with the zoo-anthropogenic activities conducted in the habitats where these plants grow has led to obvious changes both in terms of anthesis and of covered area, some of them becoming invasive. Information regarding the species in the latter category is particularly important, as it may contribute to their future management.

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DISTRIBUTION OF INVASIVE SPECIES *METCALFA PRUINOSA* IN GREEN FOREST OF WESTERN ROMANIA

Denisa-Daliana SFÎRCULUS, Ioana GROZEA

University of Life Sciences "King Mihai I" from Timișoara,
119 Calea Aradului, Timișoara, Romania

Corresponding author email: ioana_entomol@yahoo.com

Abstract

The Green Forest around Timișoara is home to numerous species of harmful insects. We would like to focus our attention on the species Metcalfa pruinosa known as Citrus Flatid Planthopper through this work. Uncontrolled, it is clear that the species can become dangerous for both woody plants in forests and those that constitute ground vegetation. Our study included observations of the size of nymph groups through the specific wax secretion left on the leaves of deciduous trees, 60-80 years old, but also older. The analysis was carried out in 7 sectors of the forest and had macroscopic techniques on site, by marking the target trees. All 8 plant species analyzed were affected in percentages ranging from 4.5% to 38%. The results showed that in sectors with predominant plants of Ulmus sp. and Acer sp. there were the most foliage with secretions, nymphs and adults. Also, the inner part of marginal trees and those near wide, airy paths were more affected by Metcalfa colonies. Regular monitoring could lead to reduction of damage.

Key words*: Metcalfa pruinosa, pest, Green Forest, trees, monitoring.*

INTRODUCTION

Metcalfa pruinosa, commonly known as the citrus flatid planthopper (CFP), is considered an invasive species in Europe (IUCN, 2000; Strauss, 2010; Forest Pests Europe website, 2024). Its origin is unclear, but it is known for sure that the first reports were made a long time ago, in North and Central America (Ontorio, Florida, Mexico and Cuba) (Metcalf and Bruner, 1948).

It entered Europe much later, in 1979, northern Italy (Zangheri & Donadini, 1980) and then gradually spread in entire continent (Pantaleoni, 1989) so that it was observed also in Romania for the first time in 2009 (Preda and Skolka, 2009). It was also reported in Asia, first in Korea (Kim et al., 2014).

Interestingly, it has established itself in various habitats in Europe, including forests. It is very polyphagous, feeding on a wide range of plants (330 species of woody and herbaceous plants) (Wilson & Lucchi, 2000), from various categories such as ornamental plants in parks, fruit trees, vines, vegetables, forest trees (Pons et al., 2002; Moosbeckhofer et al., 2012; Gogan and Grozea, 2013; Pencheva & Yovkova, 2016; Tancik & Seljak, 2017; Dobrin et al., 2020; Muntean & Grozea, 2021).

Its feeding habits can affect the general health of plants. Thus, through direct damage (feeding by extracting cell sap), photosynthesis is affected and implicitly growth and development (Vlad & Grozea, 2016).

Through indirect damage, i.e. by vax white secretion and "honeydew" by nymphs, the last sticks to leaves, fruits or other vegetative organs and can cause inhibition of essential processes (respiration and photosynthesis of leaves). In addition, a qualitative depreciation of the leaves is also created (Chireceanu & Gutue, 2011).

Most of the time, the basic symptoms are confused with secondary symptoms, so the presence of waxy filaments on the plant organs, or honeydew are considered the main symptoms in recognizing the species through the attack (Gogan & Grozea, 2013; Świerczewski et al., 2022).

It is assumed that honeydew would be eliminated depending on the body size, especially L4, L5 and adults eliminate appreciable quantities, especially in late summer (Kahrer et al., 2009).

Its adaptability allows it to thrive in diverse environments, from grassy areas, shrubs to mixed deciduous forests. The association of the CFP pest with forest habitats is often highlighted at a significant level. Studies have shown that

the cicada is frequently found in forested areas. A weighting of the host plant categories was found as, for example, in South Korea approximately 33.8% of *Metcalfa pruinosa* populations were recorded in forests (Lee et al., 2019).
The occurrence and dispersal of CFP are closely linked to a number of factors such as meteorological conditions, geographical conditions, biological factors, human activities and the availability of host plants (Pantaleoni, 1989, 1989; Kill et al., 2011).
The impact that this flatid species has on host plants can be viewed depending on the type of habitat (agro-horti-ecosystem or natural ecosystem), in many cases being underestimated (Derim, 2018).
The multiple factors contributing to the spread and presence of the CFP pest in forests are of concern with impacts on biodiversity (Byeon et al., 2017).
Given its ability to spread widely and adapt to new host plants, continuous monitoring of susceptible areas is essential for managing the impact of PCF in forested areas. Therefore, through this work we would like to bring to attention the current distribution of the foliar pest in a mixed deciduous forest through the symptomatology and characteristics but also the presence of active stages.

MATERIALS AND METHODS

Characterization of the study site

The on-site observations were made in a natural recreational forest type area, called "Padurea Verde" (Engl. "Green Forest"), which is located in the western part of Romania and belongs to the municipality of Timisoara (Figure 1). It covers an area of 560.48 ha and is composed of both deciduous and coniferous trees. Deciduous trees are predominant (560.03 ha) while conifers have a low share (0.45 ha). This forest is inhabited by the Behela River, which supplies water to the plants. Oak is the predominant species among deciduous trees, but other species such as ash, acacia, field sycamore, elm and walnut are also present (Achim & Buzatu, 2018).

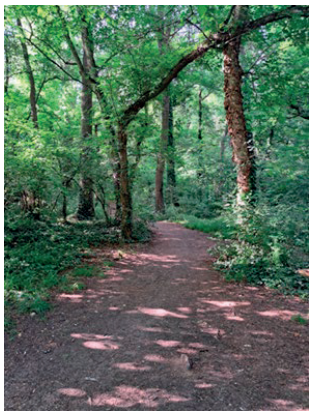


Figure 1. Place of study, the deciduous forest in Western Romania known as "Padurea Verde" (landscape image - indoor alley)

Observation points

Our attention was directed to the marginal sectors in the southern part of the forest that are located next to public roads and constructions. Thus, 7 sectors (according to the division made by the Timisoara Forest District in 2018) of this section were subjected to light visual observations, directly on site 2 times a month (July-September), during 2024. With the help of GPS, the observation points were marked (2.85 points/sector), a total of 20 points subjected to observations (Figure 2) (Table 1).

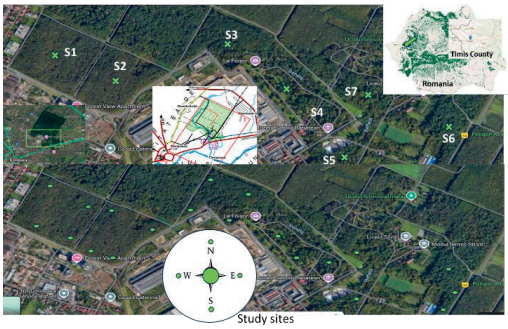


Figure 2. Marking study sites within the marginal sectors of the southern part of the forest

In some sectors we marked 4 points, in others 3 and in others 2 depending on the surface and shape. Around the observation point, approximately 2 m was measured radially (as appropriate) in the direction of the cardinal

points (N, S, E and W) and to include 4 trees each (Figure 2).

Table 1. Technical characteristics of the sectors under study

Sections	Observation points	Identification date
Section 1	P1	45.783122, 21.247595
	P2	45.783067, 21.247571
	P3	45.781965, 21.250220
	P4	45.780230, 21.248703
Section 2	P5	45.779468, 21.250372
	P6	45.781584, 21.251737
	P7	45.780568, 21.253831
	P8	45.779150, 21.251646
Section 3	P9	45.781754, 21.257048
	P10	45.783572, 21.258683
	P11	45.782682, 21.260775
	P12	45.781213, 21.259337
Section 4	P13	45.780028, 21.262335
	P14	45.778917, 21.264083
	P15	45.778378, 21.266115
Section 5	P16	45.775585, 21.266365
Section 6	P17	45.775794, 21.273754
	P18	45.776674, 21.274223
	P19	45.778235, 21.273754
Section 7	P20	45.779519, 21.268485

The leaves on the lower level of the tree crown were observed (sometimes using a mobile foldable ladder) and were directly analysed. The leaves with white secretions on the underside (specifically for *Metcalfa* nymphs) were counted, as well as the nymphs inside the secretions and the adults (known to be good jumpers) where they were observed. Images were taken by video and photos then quantified on electronic material enlarged on the computer. This method was adapted for this type of ecosystem (natural) so that the leaves with pests and damage present would continue to remain on the tree.

Indirect symptomatology (white secretion) produced by the nymphs of *Metcalfa pruinosa* can be easily confused with that of the woolly aphid (*Eriosoma lanigerum*), but the distinction between the two can be made on the spot. That is; to identify the woolly aphid, high density of secretion and it's much more robust consistency are considered (Palagesiu et al., 2000).

Grassy vegetation on the ground or on the tree trunk was also observed. Additionally, the levels of secretions on them and the presence of nymphs and adults were taken into account. The reason was based on the potential migration onto the leaves of the tree under analysis.

No leaf was detached from the tree regardless of the degree of attack.

Where applicable, raw data were statistically interpreted (Anova test, descriptive statistics).

This type of study is one of observation and warning about the future danger of population growth and direct and indirect damage.

RESULTS AND DISCUSSIONS

Our observations showed that on the 8 species of plants marked (ash, acacia, elderberry, elm, hornbeam, maple, oak, walnut) the CFP species as a phytophagous pest was present in various percentages on leaves and shoots. Among these tree species, the elder is considered a small tree or shrub (like elderberry). Most are tall trees but even so the pest presence was reported in active forms (nymphs and adults). Although oak is the predominant species in the Green Forest, the phytophagous pest (*Metcalfa*) was present in a diversified manner on the monitored trees.

From what was observed on the active stages, nymphs and adults were present on all tree species, more precisely the leaves and shoots of the trees in various sizes. The highest numbers of nymphs in all sectors and observation points (P) taken as a whole were quantified on field sycamore (with an average of 6.8/leaf), elm (4.5 nimph/leaf), oak (average of 4.0) and ash (average of 3.0). Considered to be of small size were the average levels seen on elder and hornbeam (2.6 nymphs/leaf), acacia (average of 1.0) and walnut (0.5) (Figure 3).

Adults were also present on all 8 tree species, smaller than nymphs and observed only on shoots. There were differences in values between them, so the most were observed on elm (with an average of 2.8 ind./shoot) and sycamore (2.0 ind./shoot) and on oak (1.9 ind./shoot). Fewer were observed on ash and hornbeam (with an average of 1.5 ad./shoot), acacia and elder (0.5 ind./shoot) and walnut (0.2 ad./shoot) (Figure 3).

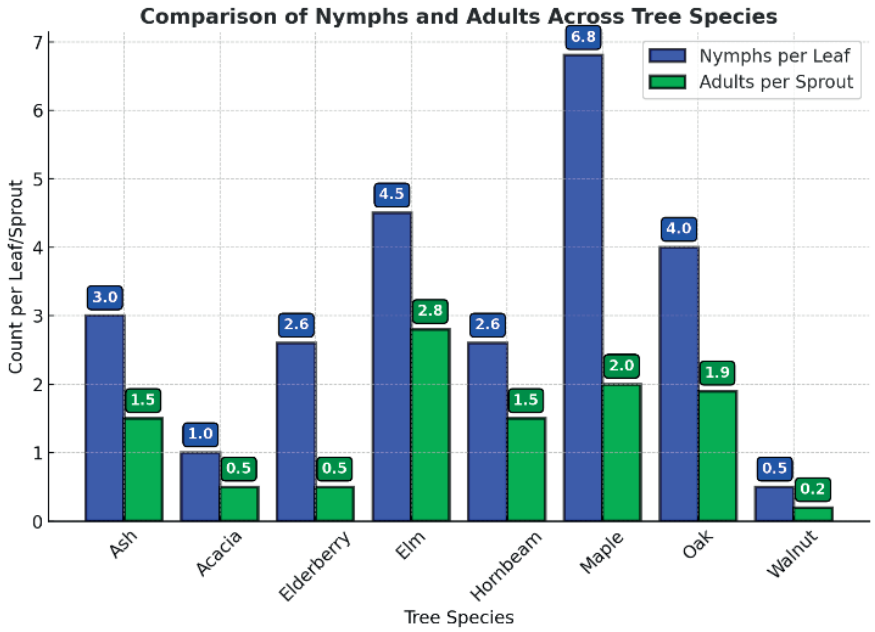


Figure 3. Chart comparing the average number of nymphs per leaf and adults per shoot from different tree species

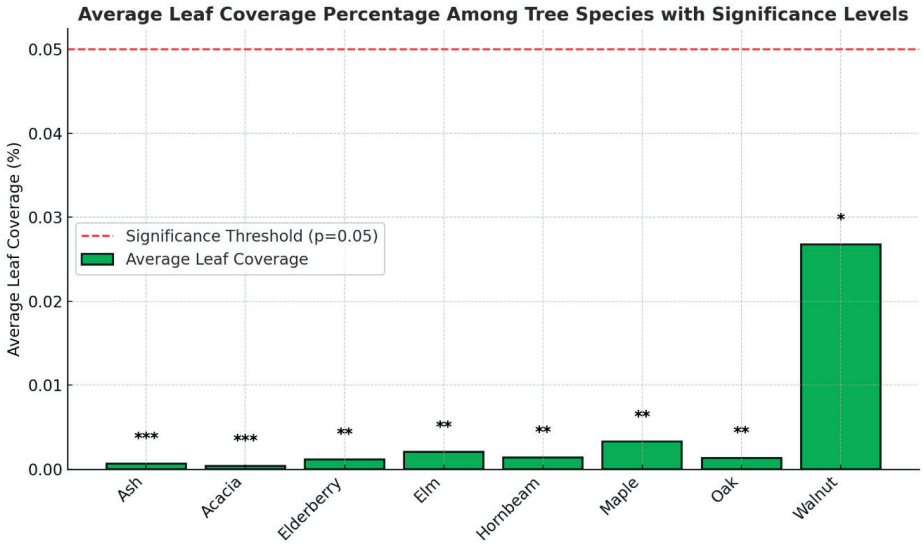


Figure 4. Diagram comparing the percentage of leaves covered with secretions produced by *Metcalfa pruinosa* nymphs (out of a total of 50 leaves analyzed/each tree)

A descriptive analysis of these without taking into account the observation points and months shows average values of nymphs per leaf of 3.13 and standard deviation (StD) of 2.0 and of adults per sprout 1.36 with Std of 0.9 and range (R) of 0.5 - 6.8 and of adults per sprout 1.36 with Std of 0.90 with R of 0.2 - 2.8.

All species have statistically significant differences from the mean, namely walnut is statistically significant (*) ($p<0.05$), oak,

elderberry, elm, hornbeam and maple very statistically significant (**) ($p < 0.01$) and ash and acacia extremely statistically significant (***) ($p < 0.001$) (Figure 4).
Considering the percentage of leaves covered with nymphal secretions from 50 leaves (Figures 4, 5) analyzed over 3 months (July, August, September) we found that all trees had positive mean values of nymphal secretions on leaves, in percentages varying from 2% to 38%, with maple and elm predominating with 20 and 33% respectively, but also hornbeam, oak and elderberry with 14, 13 and 12%. Ash, acacia and walnut had mean values lower than 8% (7, 4 and 2%).



Figure 5. a) Nymphal colonies and fine white filamentous secretions on the underside of leaves (woody vegetation); b) details of the damage and nymphs present on the leaf petioles and leaves; c) d) adults on shoot

Monthly percentage of damage without considering observation points and tree species revealed an increasing trend in values, with an average of 8.9% in July, 11.9% in August and 13.0% in September (Figure 6).
Standard deviation shows an increase in the variation of damages from one month to another (July: 5.46%, August: 7.29%, September: 8.01%), suggesting that the differences between the observation points increased during the three months analyzed. Minimum value of damages is very small (0.1%-0.5%), indicating the presence of observation points with minimal or almost non-existent damages.

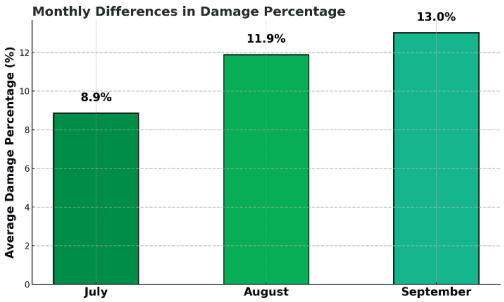


Figure 6. Graph showing the monthly percentage of damages without taking into account the observation points

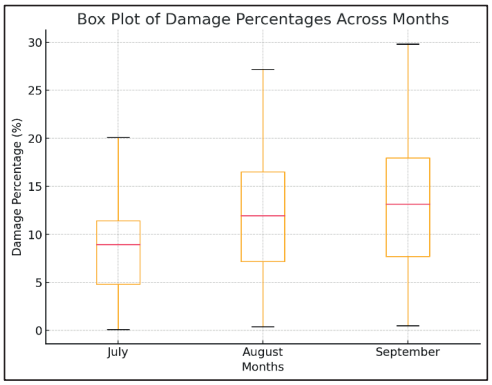


Figure 7. Boxplot diagram of damage percentage for the period June-October

Maximum value of damages is significantly higher in September (29.8%) compared to July (20.1%) and August (27.2%), signalling that over time certain points suffered a considerable increase in damages. Quartiles confirm the increasing trend, showing that most points suffered increasing damages, especially those in the upper part of the distribution (75%) (Figure 7).
Observation points vary significantly in terms of the average damage recorded, with both areas with minimal damage and areas that were severely affected (Figures 8, 9).
Overall average damage is approximately 11.26%, which shows that, in general, the points recorded a moderate level of damage (Figure 8).

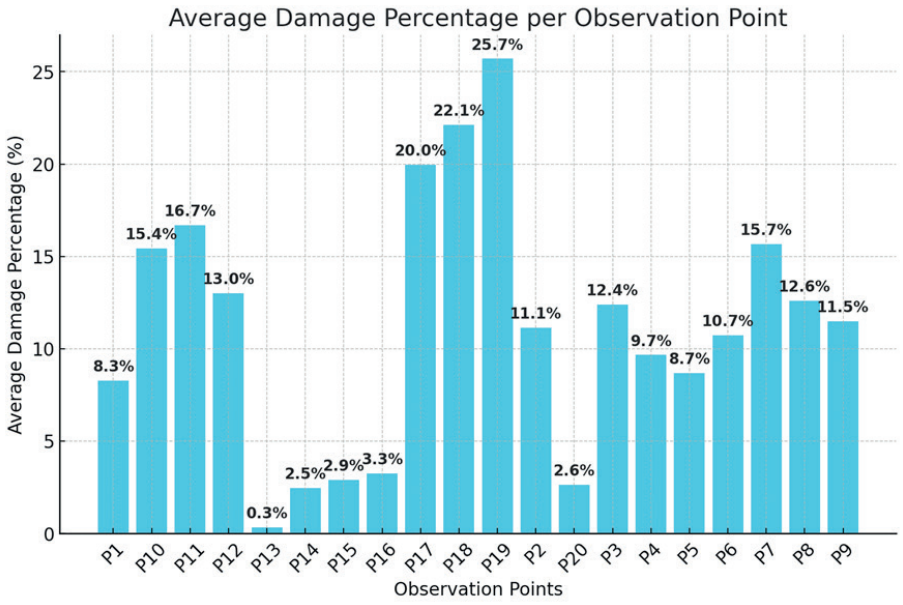


Figure 8. Chart comparing the average percentage of damage for each observation point (P) without considering months

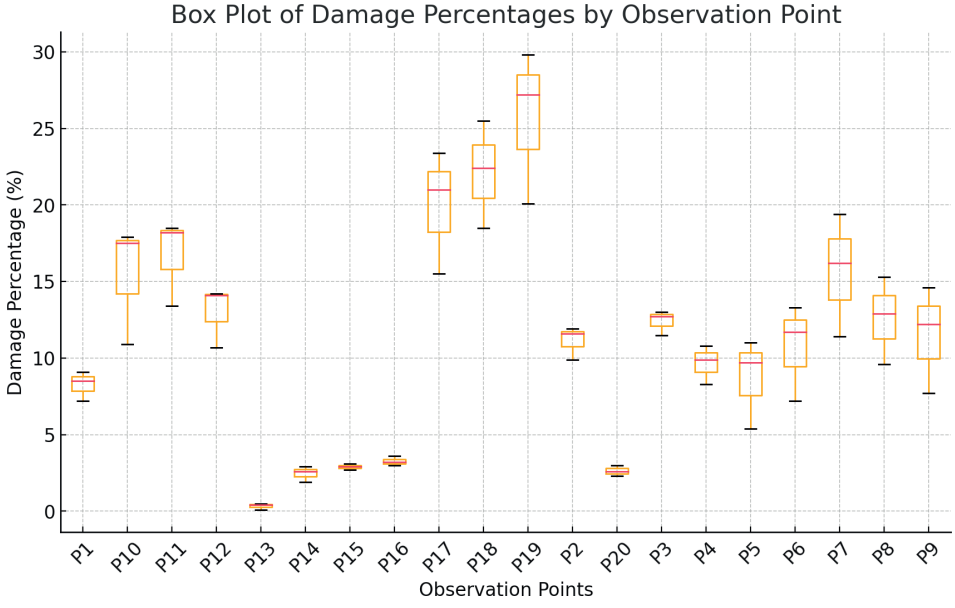


Figure 9. Box plot comparing damage percentages across observation points; viewing medians and potential outliers for each point

The standard deviation of 6.88% indicates a significant variation between the observed points, which suggests the existence of different conditions that influence the damage. Minimum value of the average damage per observation point is very low (approximately 0.33%), highlighting the existence of points with minimal or insignificant levels of damage. The maximum value of the average damage (25.7%) shows that there are observation points with

high damage, well above the overall average. The quartiles suggest that half of the observed points have average damage below 11.32%, while a quarter of the points recorded damage above approximately 15.49%.

CONCLUSIONS

As a result, in the marginal sectors of the southern part of the Green Forest in Timisoara, both nymphs and adults of *Metcalfa pruinosa* were evident on the leaves and/or shoots, on the one hand, but also white secretions on the leaves. All monitored tree species had leaves with direct and indirect attack that disrupted the proper functioning of deciduous trees. The evolution of the damage in 2024 was progressive and is expected to increase in the following years in the absence of a strategy to keep it under control. We suggest the use of traps in future monitoring activities to assess the evolution of the damage.

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CREATING A CLIMATE CAMERA FOR GROWING MICROGREENS: DESIGN, PARAMETERS AND POSSIBILITIES FOR APPLICATION

Hristo TANKOV, Manol DALLEV, Dimka HAYTOVA

Agriculture University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: manol_dallev@abv.bg

Abstract

Microgreens are a new category of specialized vegetable crops that are included to the concept of fresh functional foods. The increased interest in this type of products has focused producers on the search for technological ways to produce them. One way to provide a practical application based decision is through the use of a climate camera. The creation of a climate camera with precise control of the growth and development conditions of microgreens is a key element of their production technology. This publication discusses the parameters necessary for optimal growth of microgreens. It describes the basic principles for the design of a climate camera for their cultivation. As a result of the review, it presents practical guidelines for successful design and application. The main conclusion is, the design of a climate chamber for microgreens is based on the correct choice of materials and creating the opportunity to control the main growth factors - temperature, humidity and light.

Key words*: microgreens, growth, climate control, smart box design.*

INTRODUCTION

Microgreens, or micro-plants, represent a rapidly developing field in agricultural and biological sciences. These plants, grown at an early stage of development, are used for various purposes – from scientific research to commercial production. Creating a climate chamber with precise control of conditions is essential for optimizing their growth. The chambers must provide a stable and controlled environment to maintain consistent growth and development of the microgreens. Growing micro-plants in a climate chamber is an innovation that is entering Bulgaria with great potential for development in the field of sustainable agriculture.

Microgreens are young plants grown in the early growth phase (usually 7-21 days) that are rich in nutrients, vitamins, minerals, and antioxidants. Studies show that they can contain significantly higher levels of nutrients compared to fully developed plants (Xiao Z. et al., 2012). Thanks to their health benefits and appealing appearance, they are widely used in culinary arts, functional nutrition, and dietetics. In Bulgaria, although interest in microgreens is growing, this is still a relatively new direction in the agricultural industry. The use of climate chambers for their cultivation allows precise control of conditions such as temperature, humidity, and lighting, which significantly accelerates growth and ensures a higher yield per area. With advances in agronomy and technology, climate chambers are becoming increasingly popular and provide new opportunities for farmers in Bulgaria to take advantage of the rapidly growing market for microgreens.

However, traditional methods of cultivation in greenhouses or indoors are often limited by factors such as seasonality, climate change, and high energy costs(Chiruta et al., 2024; Hoza et al., 2024; Iovita et al., 2024).

Creating a climate chamber that can provide perfectly controlled conditions for growing microgreens is of utmost importance for modern urban agriculture and vertical farming. Climate chambers offer a controlled environment in which key parameters such as temperature, humidity, light, and carbon dioxide (CO_2) concentration can be adjusted. These conditions allow optimization of the growth and quality of microgreens, such as regulating the light spectrum and intensity, which affects photosynthetic activity and the accumulation of bioactive compounds (Kyriacou M. C. et al., 2016) and (Samuolienė et al., 2013). Maintaining optimal levels of CO_2 is also essential for stimulating plant growth and development (Stutte G. W.,

2006). Such a chamber would allow year-round production, minimizing losses and optimizing the quality of production.
The aim of the present study is to design and investigate a climate chamber for growing microgreens. This chamber will be developed with a focus on the following parameters:
1. Temperature: Precise control for optimal growth.
2. Humidity: Precise control for optimal growth.
3. Light: Regulation of the spectrum and intensity of lighting.
In addition, the article will discuss the possibilities for the application of the climate chamber in urban farming and the industrial production of microgreens.

MATERIALS AND METHODS

The construction of a climate chamber for growing plants requires careful planning and understanding of several key elements that provide the right conditions for growth. The main steps are the selection of size and materials for the construction of the chamber.
The size of the climate chamber depends on its intended use – whether it will be used for scientific experiments, industrial production, or personal needs. The main factors that determine the dimensions are the number of plants and the type of crops that will be grown in it. The chamber should have enough space for free growth of the crops. For example, for small experimental plants, it may have a volume of 0.5–1 m³, while for industrial production it can reach several cubic meters. In our case, we are creating a chamber with internal dimensions (Figure 1) of 0.7 x 0.5 x 0.5 m with a volume of 0.175 m^3 intended for experimental and personal needs. The main goal for choosing this size is the mobility and the need to be easily portable with the possibility of installation on terraces and in small rooms.



Figure 1. Chamber with internal dimensions of 0.7 x 0.5 x 0.5 m with a volume of 0.175 m^3

The choice of materials is essential for maintaining optimal microclimate and efficiency of the chamber. Studies have shown that the most commonly used materials are:
• Polycarbonate panels – Lightweight, impact-resistant, and with good thermal insulation. They allow some light transmission.
• Aluminum or steel frame – Provides strength and stability to the structure.
• Chipboard or plywood with thermal insulation – A good option for budget chambers with a fixed location.
• Polyurethane foam – Provides additional thermal insulation.
Extruded polystyrene foam (XPS) (Figure 2), or its common name "Fibron", was used for insulation.

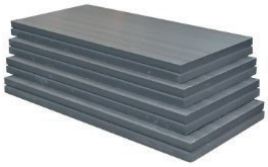


Figure 2. Extruded polystyrene foam (XPS)

The material is suitable for the production of floor, roof, and wall insulation. XPS offers very good thermal insulation with a thermal conductivity coefficient (λ) of about 0.029-0.035 W/mK. This makes it effective in preventing heat loss. Its density varies between 25 and 45 kg/m³, depending on the manufacturer and intended use. Compressive strength under pressure at 10% deformation (20-40 kPa) depending on the type, but can reach 300 kPa for specific applications. Covered with a closed-cell structure, XPS is resistant to moisture and does not absorb water, making it vapor-tight and ideal for applications where moisture control is important, and with water absorption of less than 0.5% it is waterproof. Due to the chemical composition of the material, XPS is not subject to rot or mold growth. The box is lined on the outside with fiberboard and edged with PVC profiles for greater structural strength. Fiberboard is a composite material composed of wood fibers (Figure 3) bonded with adhesives, which is widely used in construction, furniture manufacturing, and interior design. Its density is usually between 600 and 800 kg/m³. It is available in various surface treatments, including lamination, foil, melamine coating, or

natural wood. It provides good thermal and sound insulation, making it suitable for lining the climate chamber.



Figure 3. Fiberboard - composite material composed of wood fibers

For internal coating, the most commonly used materials are:

- Reflective film (Mylar) – Increases lighting efficiency by reflecting light back to the plants.
- PVC or aluminum foil – Easy to clean and moisture resistant.

Our choice is mirror window film. The mirrored part (Figure 4) is oriented towards the plants in order to reflect the emitted light towards the cultivated crops.



Figure 4. Mirror window film - the mirrored part

The main climatic factors that need to be managed include temperature, humidity, lighting, ventilation, and carbon dioxide (CO_2) concentration. Adjusting these parameters is key to creating optimal conditions for growing plants in laboratory and industrial settings.

Temperature Control

Temperature affects photosynthesis, respiration, and other physiological processes of plants. It is usually maintained in the range of 18-30°C.

Heating: Heating elements provide the necessary temperature, especially during the winter season (Liu et al., 2000). To ensure effective temperature control in the climate chamber, a system consisting of an electric heater and a fan that draws air from the external environment is often used. This configuration is assembled in a metal cylinder and allows maintaining optimal temperature conditions for plant growth by evenly distributing heat. At the end of the module, a V-shaped valve is mounted to prevent heat loss from the chamber to the environment. An electric heater with a supply voltage of 12V and a power of 110W (Figure 5) was used.



Figure 5. Electric heater with a supply voltage of 12V and a power of 110W

The fan is a 9CFM FD05015S, SPIRE, 4800RPM, 50x50x15mm, 12VDC with a flow rate of 15 m^3/h.

The configuration is mounted on the upper side edge of the chamber. The temperature is regulated by a programmable thermostat XH-W3002 designed for automatic switching on and off of the load at specific temperatures, equipped with a temperature sensor, model W3002 Digital LED Temperature Controller 12V/10A (Figure 6).

- Regulation range: -55 to +120°C.
- Temperature regulation accuracy: 0.1°C.
- Temperature measurement accuracy: 0.2°C.



Figure 6. Temperature sensor, model W3002 Digital LED Temperature Controller 12V/10A

Cooling: Cooling systems in climate chambers are essential for maintaining a stable temperature, preventing overheating, and creating

optimal conditions for plants. Depending on the scale of the chamber, the accuracy of control, and the budget, different types of cooling systems can be used. Air conditioning systems and fans regulate the temperature in warm conditions (Katagiri et al., 2015). The most commonly used temperature control systems are:

- Air cooling (fans and air exchange), fans. This is the simplest and most economical system, which is used to reduce the temperature by circulating air.
- Cooling with a compressor air conditioner - climate chambers with a built-in refrigeration compressor operate on the same principle as ordinary air conditioners - by circulating a refrigerant (freon).

Based on the conducted study, according to the volume and temperature requirements in the presented chamber, a system including Peltier elements with integrated water cooling of the hot part was manufactured and installed for the cooling system. Peltier modules (Figure 7) (thermoelectric coolers) generate a significant amount of heat while they operate. If this heat is not dissipated effectively, their cooling capacity decreases. Water has a much higher thermal conductivity (0.6 W/m·K) compared to air (0.026 W/m·K), a specific heat capacity of 4.2 kJ/kg·K, which means it can absorb more heat per unit mass without heating up significantly. Water cooling uses a radiator with a fan and circulating liquid, which means that heat is distributed more efficiently than with air cooling. The fans that blow the cold part of the module also help to evenly distribute the temperature in the box.



Figure 7. Peltier modules

The temperature is regulated by a programmable thermostat XH-W3002 designed for automatic switching on and off of the load at specific temperatures, equipped with a temperature sensor, model W3002 Digital LED Temperature Controller 12 V/20 A. The total power of the module is 144 W.

Air intake fan. The fan provides air circulation in the chamber and brings in fresh air from the external environment. Axial or centrifugal fans with a power of 5 W to 50 W are used, depending on the volume of the chamber. In our case, a fan model Sunon (Figure 8) EF92251S3 12VDC, 92 x 92 x 25 mm, 1.32 W, sleeve, 87.04 m³/h was used.



Figure 8. Fan model Sunon EF92251S3 12VDC

To ensure optimal air circulation in the climate chamber, the fan is controlled by a time relay. This system allows periodic switching on and off of the fan, thus maintaining a uniform distribution of temperature, humidity, and CO_2 in the chamber.

Lighting and Photoperiod: Plants need adequate light for photosynthesis, and the spectrum and intensity of lighting must be tailored to their needs. The types of artificial light sources are:

- **LED lighting**: Allows adjustment of the spectrum to stimulate growth and flowering (Poorter et al., 2016).
- **Fluorescent lamps**: Suitable for laboratory conditions, but with less energy efficiency.
- **Sodium lamps**: Often used in greenhouse conditions.

For the purpose of the study, the most affordable waterproof LED strip with blue and red light was chosen. The distribution of diodes is 5:1 red to blue.

- LED type: SMD 5050
- Number of LEDs: 60 LED/m
- LED wavelength: Red: 660 nm; Blue: 450 nm
- Voltage: DC 12 V
- Power: 8 W/m, 36 W/5 m

A module (Figure 9) was made consisting of 9.5 m of the above-mentioned strip, attached to an aluminum plate with dimensions... with a

mounted aluminum radiator to dissipate the heat generated by the LED strip. The amount of light delivered to the plants is 1010 lx. Lux (lx) is a unit of measurement for illuminance, which describes the amount of light falling on a surface. One lux is equal to one lumen per square meter (l m/m²).

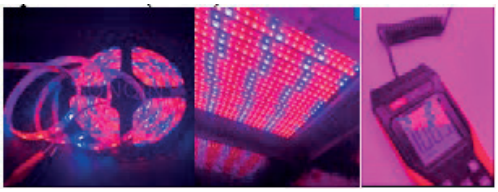


Figure 9. Waterproof LED strip with blue and red light

Plants have two main pigments that absorb light for photosynthesis:

- **Chlorophyll A**: Absorbs blue light best around 430 nm and red light around 662 nm.
- **Chlorophyll B**: Absorbs blue light around 453 nm and red light around 642 nm.
- **Blue light (450-470 nm)**: Stimulates vegetative growth - enhances the formation of leaves and stems, improves branching and makes plants more compact. It helps in the development of the root system.
- **Red light (620-680 nm)**: Accelerates flowering and fruiting, supports leaf expansion, increases the production of flowers and fruits.

The photoperiod is 12/12 h, controlled by a time relay T2310 (Figure 10) DC 12V which allows timer modes of 000-999 sec., 000-999 min., 000-999 hours. Times can be combined (example: working in seconds and off position in hours).



Figure 10. 12 Volt Time relay – T2310

RESULTS AND DISCUSSIONS

The climate chamber is designed to provide stable conditions for growing microgreens. The temperature conditions in the chamber are maintained with stable heating via a module with a fan and heating element, which provides minimal fluctuations (around 0.4°C) around the desired temperature of 23.5°C. The heating works stably even at external temperatures in February from -5°C to 12°C during the day and internal room temperatures from 2°C to 12°C. The hysteresis of the thermostat (switching on at 23.1°C and switching off at 23.5°C) ensures minimal variations. Cooling with Peltier elements is not activated under these conditions, as the external temperatures are not low enough to cause the need for cooling in the chamber.

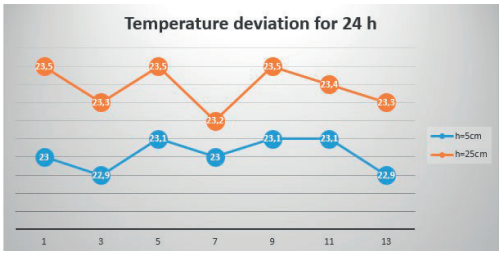


Figure 11. Fluctuations in temperature over time at different heights

When measuring the temperature at different levels in the chamber, it was found that at the bottom it is 23°C and at a height of 25 cm it is 23.5°C, where the are located the top of microgreens (Figure 11). This indicates small fluctuations in temperature at different heights, which are due to heat circulation and air distribution. The air circulation in the chamber is provided by a 12-volt fan with a flow rate of 42 cubic meters per hour, which ensures even distribution of temperature and prevents the accumulation of hot zones.

The humidity in the chamber was monitored with a hygrometer, with normal humidity being around 26%. After watering, the humidity increases to 32%, but does not remain at higher levels, which is typical for manual watering.

Lighting in the chamber is provided by LED strips with a power of 8W per meter, located at 9.5 meters in total. The distribution of strips with 5 red and 1 blue diode is optimized for maximum growth of microgreens. The illuminance reaches 1075 lux at a distance of 40 cm from the strips, which is suitable for plant growth. After the selected studies, selection trials were conducted with radish test plants

while observing the temperature dynamics. The experimental conditions included different combinations of the height of the seed container from the bottom of the chamber and different light intensities. At a bottom height of 50 cm and a light intensity of 1010 lux, a 5:1 red to blue ratio, the plant reached a height of 12.5 cm on the 5th day after sowing. Changing the height to 38 cm and increasing the light to 1700 lux with the addition of white light 5:1:1, on the 5th day the plants were 7 cm. The specific difference in appearance is directly related to the vitality of the plants and the commercial appearance of the finished product (Figures 12 and 13).



Figure 12. Case 1: 12.5 cm plants under 1010 lux 50 cm height lights



Figure 13. Case 2: 7 cm plants under 1700 lux, 38 cm height lights

Our preliminary results agree with the findings of Gunjal et al. (2024).

Analysis of variance (ANOVA) was conducted to establish the relationship and strength of influence of light intensity on plant growth (Table 1).

Table 1. ANOVA statistical analysis

Source of Variation	SS	df	MS	F	P-value	F crit	strength of influence
Between Groups	6740186	1	6740186	205,4	0,00	4,4	92%
Within Groups	590772,4	18	32821				8%
Total	7330958	19					

A strong correlation between light intensity and plant height was found - 92%. This relationship is described by the following linear equation $y = -79.354x + 2008.8$ (Figure 14).

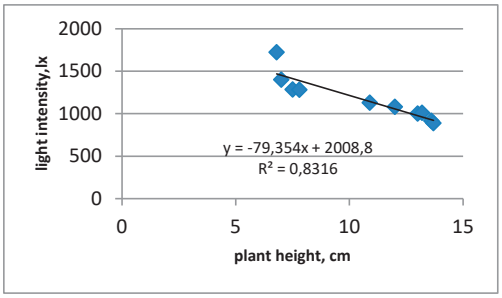


Figure 14. Correlation between light intensity and plant height

The high coefficient of determination (0.832) shows that at a light intensity of 1400 lux, the height of the plants will be 7-7.5 cm with a probability of 83%. It is statistically proven at a level of significance of 5%.

CONCLUSIONS

The present results demonstrate that the climate chamber provides stable conditions for growing microgreens at low external temperatures. The heating system maintains the desired temperature of 23.5°C with minimal fluctuations of 0.4°C, with cooling via Peltier elements not being activated under the current conditions.

Air circulation, provided by a 12V fan with a flow rate of 42 m³/h, contributes to the even distribution of temperature. Illumination of 1075 lux at 40 cm from the LED strips with an optimal ratio of red and blue diodes is suitable for the growth of microgreens.

Air humidity remains relatively low (26-32%), with a temporary increase observed after watering. This indicates a need for additional humidity management measures if the goal is to optimize growing conditions.

Based on these results, the climate chamber can successfully maintain the necessary parameters for growing microgreens. Future improvements could include automated watering, humidity control, and additional temperature measurements at different heights to further optimize the environment.

The main parameter of the climate camera for growing microgreens, which is important for the height of the plants, is the light intensity.

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RESEARCH ON THE VALORIZATION OF MELONS AND THEIR BY-PRODUCTS

Cristina TOMA (VAPOR)[1, 2], Gheorghița HOZA[1], Radu-Andrei STERIAN[1, 2], Simona POPESCU[2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]ICDIMPH-HORTING Institute, Bucharest, Romania

Corresponding author email: cristina.vapor11@gmail.com

Abstract

The efficiency of utilizing watermelon rind has made the study presented in this paper spark interest in the potential of valorising watermelons rich in citrulline in various food products, as well as addressing a management and environmental issue. Consumers are becoming increasingly health-conscious and are showing interest in various new food products. The proposed objective was to create two different products based on watermelon rind. The resulting products fall into the categories of "Jam" and "Pickled vegetable preserves (in vinegar)". Fresh watermelon rind (Citrullus lanatus) was washed, the green outer layer was removed, and the rind was cut into uniformly sized pieces. For the jam, blanched rind was used, over which syrup (water, sugar, and lemon juice) was added and left to infuse. For the vegetable mix, the raw rind was used, mixed with vegetables (carrot, celery, green tomatoes, bell pepper, celery leaves) in a vinegar solution.

Key words*: consumers, products, valorisation potential, watermelon, watermelon rind.*

INTRODUCTION

Watermelon (*Citrullus lanatus*) is widely consumed because of its health benefits and low calorie count (Dammak et al., 2019; Masih et al., 2021). Its peel is one of the major wastes. Watermelon rind mainly contains pectin-rich carbohydrates and different methods are available for pectin extraction (Edwards AJ et al., 2003; Hee-Seon Han and Kyung Bin Song, 2021).

The nutritional importance of watermelons has been studied by Dr. Eng. Stefan NANU et al. (2023) and Naz A et al. (2014) for their particularly wide range of organic substances and minerals. Watermelon is an excellent source of vitamins A, B1, B6 and C, when consumed, the citrulline inside is converted into an amino acid called arginine, which is of great benefit to the heart, liver and circulatory system and helps maintain a healthy immune system.

The fruit is generally consumed fresh, but its peel is disposed of as waste. Thus In the study of Setlhoka et al. (2024) presents us the possibility of valorization by different variants of watermelon (cv. Sugar Baby) sweetness with peel and pulp.

Also, Arivuchudar (2023) and Aṣoka s. et al. (2021) in their researches present the importance of watermelon peel, which is a source of water, carbohydrates, proteins, vitamins, minerals and phytochemicals. Based on watermelon rind, he prepared a biscuit-like snack that is popular with consumers.

Research conducted by Mubarak et al. (2022) on the utilization of watermelon by-products as ingredients for a pastille-type product with different percentages of red pulp and peel puree. They used watermelon pulp, lycopene sources and peel by-product as citrulline sources. Another research presents the preparation of melon peel candy based on sugar and honey (Thapa et al., 2022).

Another experiment was carried out at Postharvest Laboratory, Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, from February 2018 to December 2018 to determine the quality of melon peel jam with different concentration of sugar and flavor (Monir et al., 2019; Shruti Dubey et al., 2022).

Another research to highlight the potential of watermelon rind as a source of nutrients was carried out by Haque, Ahmed Redwan (2020) by utilizing them in the form of noodles.
The aim of the work was to emphasize the value of watermelon peels to obtain new and quality food products, and 2 products were obtained based on watermelon peel: watermelon peel sweet and Pickle Mix.

MATERIALS AND METHODS

The experiment was carried out at the ICDIMPH-Horting and the raw material was obtained in the institute's own greenhouses. The plant material used to set up the experiment was two watermelon hybrids: Sorento and Baronesa.
The preserves, "Watermelon rind jam" and "Watermelon rind in pickled vegetable mixture" were obtained with the help of the research team in the Research and Valorization Laboratory.
The focus of the work is directed towards the potential valorization of watermelon rinds for consumption in various preparations. Fresh watermelon peel which is a typical and unappetizing waste material, through the experimental process was transformed into new products with appreciated quality attributes.
The watermelons were analyzed quantitatively and qualitatively, sorted and washed until the moment of division. In order to create the product Sweetness according to the scheme in Figure 1, they were peeled of the green peel and red core. The watermelon rinds were washed, divided, blanched, allowed to diffuse in the syrup and then concentrated as shown in Figure 2. An important step in the technical flow is the conditioning of the containers. The resulting product is dosed into the prepared containers, closed and pasteurized at 100°C for 15 minutes. The preserves are labeled and stored under appropriate conditions, see Figure 1.
Pickles are a traditional product for the Romanian market, being a staple product and account for about 90% of the preserves obtained by natural acidification (Adriana Păucean, 2011).
To explore the possibilities of valorization of melon peels were used in a pickle mix. Thus the melon peel, was peeled of the green part and the red core.

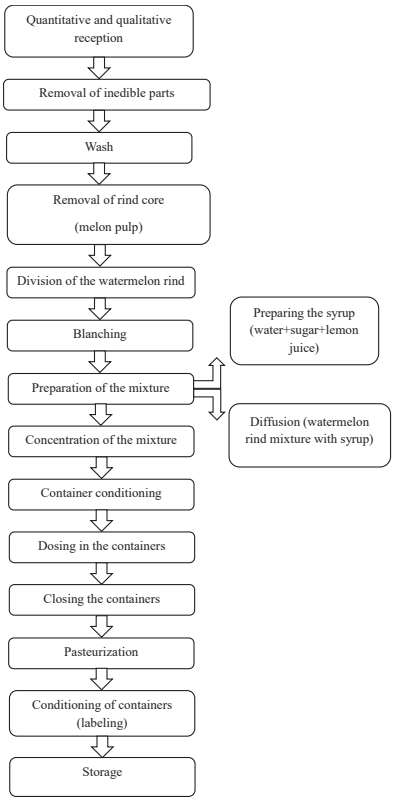


Figure 1. Technology flow of the product (Jam)



Figure 2. Stages in the technological product flow (Jam)

According to the established ratio (solid/liquid) the peel was divided into cubes and added to the vegetable mix. In the presented technological process the following steps are highlighted: conditioning of the containers, dosing of the product and filling with vinegar solution, sealing, pasteurization, labeling and storage according to the scheme shown in Figure 3.

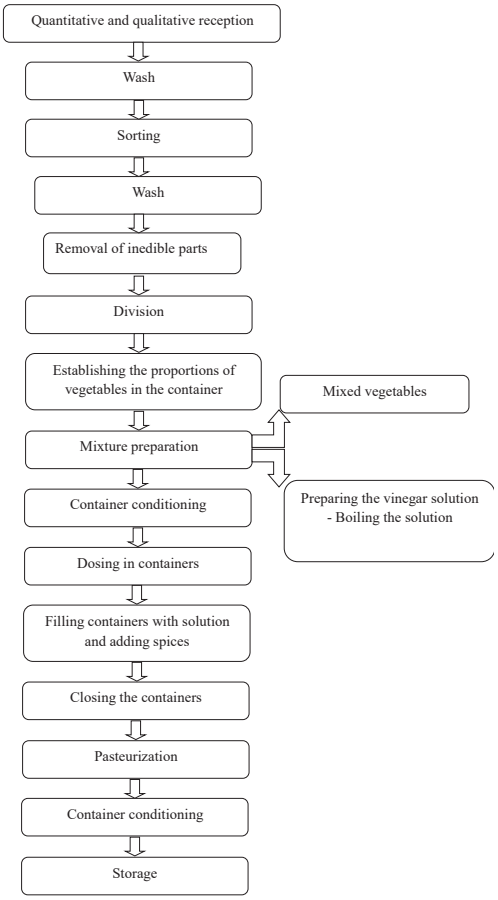


Figure 3. Technological flow of the product - Pickles

Figure 4 shows the split watermelon rind, the vegetables used in the pickling mix and the labeled finished product.



Figure 4. Stages in the technological product flow -Pickles

The obtained products were presented for tasting to the staff of the other departments of ICDIMPH Horting (Figure 5), based on a tasting sheet with scores from 1-5 for the analyzed characteristics (1 - inadequate, 2 – mediocre, 3 - acceptable, 4 - good, 5 - very good).

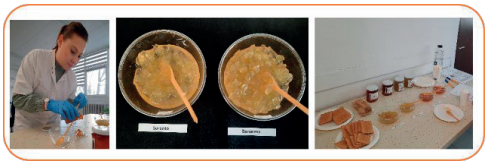


Figure 5- Tasting products

Sensory analysis allows better identification of the organoleptic characteristics of the products and consumer preference.

RESULTS AND DISCUSSIONS

Between the two hybrids analyzed there were differences in the yield of utilization of melon peel and core, the hybrid Baronesa stands out from the hybrid Sorento (Figure 6).

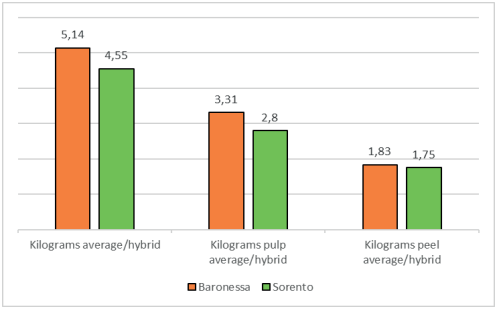


Figure 6- Yield of watermelons

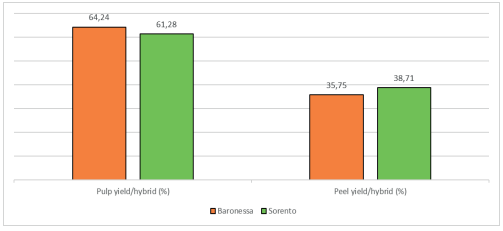


Figure 7. Shell and pulp yield

The amount of fresh by-product ranged from 35.75% in hybrid Baronesa to 38.71% in hybrid Sorento of the original weight (kg) (Figure 7).

Quality control contributes to product development according to consumer preferences. The products “Sweetness” and “Pickle mix” were tasted and appreciated by

the consumers and the result is shown in Figures 8 and 9.

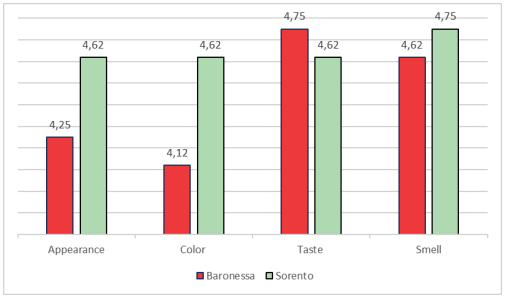


Figure 8. Results obtained during the tasting of the product - Jams

Figure 8 shows the appreciation of the sweetness of the hybrids obtained from the Baronesa and Sorento hybrids in terms of organoleptic characteristics. The sweetness from the rind of hybrid Sorento watermelon was more appreciated in terms of appearance, color and smell compared to the sweetness obtained from hybrid Baronesa. In terms of taste, the sweetness obtained from the Baronesa hybrid was more highly rated, with a score of 4.75 compared to 4.62 for the sweetness obtained from the Sorento hybrid.

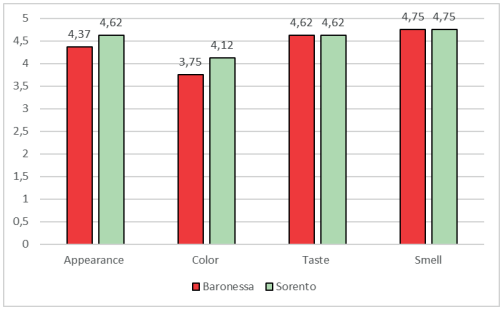


Figure 9. Results obtained from tasting the pickle mix

As regards the pickled vegetable mix analyzed organoleptically, it was found to have the following characteristics:
- appearance: relatively uniform, mosaic-like
- consistency: the split melon rind is penetrated by the vinegar solution, undistintegrated and firm,
- the taste and smell is typical of pickled vegetables.

The values obtained between the two hybrids are close, because the same spiced vinegar solution and the same ratio of divided vegetables were used.

The analysis of the tasting sheet showed that the product obtained from the peel of the Sorento hybrid was more highly valued, with higher scores for smell (4.75), taste (4.62), color (4.12) and consistency (4.62) than the product obtained from the peel of the Baronesa hybrid, which scored higher for smell (4.75), taste (4.62), color (3.75) and consistency (4.37) (Figure 9).

CONCLUSIONS

Based on the present study, it was found that a perishable and seasonal raw material can be efficiently valorized by processing and end products appreciated by consumers can be obtained.

Fruit quality influences the ratio between the pulp of melons and the quantity of by-product, the yield in processing being a characteristic of the variety;

The opportunity of valorizing the melon peel from Sorento and Baronesa hybrids by processing both sweet and pickled products was highlighted.

The final products obtained were appreciated differently by the consumers, most of them appreciating those obtained from the peel of the Sorento hybrid more highly.

The products obtained from watermelon rind (jam and pickle mix) can be classified under the categories "Jam" and "Acidified vegetable preserves (in vinegar)".

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