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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING

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MORPHOLOGICAL TRAITS OF SOME *LONICERA* SP. VARIETIES AFTER FIRST YEARS GROW IN ROMANIA

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Abstract

The blue honeysuckle (Lonicera caerulea L.) is a perennial fruit-bearing shrub that originated from distant Siberia and northeastern Asia. The objective of this study is to present the morphological traits of 16 varieties of Lonicera sp. from a young orchard established in micro-tunnels and 3 varieties in an open field planted in 2023 at distances of 3.0 × 1.0 m. The characteristics of each variety were analyzed in detail, including height, crown shape, leaf parameters, branch structure, as well as flowers and fruits distinctive traits. The research was conducted in the experimental field of the Faculty of Horticulture - University of Agronomic Sciences and Veterinary Medicine of Bucharest and Research Institute for Fruit Growing Pitești – Mărăcineni involving varieties of Lonicera caerulea and Lonicera kamtschatica planted in the spring - autumn of 2023.

***Key words**: Lonicera caerulea, Lonicera kamtschatica, morphology, phenology, traits.*

INTRODUCTION

Blue honeysuckle, scientifically known as *Lonicera caerulea* L., is a member of the Caprifoliaceae family. It goes by various names such as "haskap", "sweet berry honeysuckle", "edible honeysuckle", "Kamchatka berry" and "honeyberry", as mentioned in previous papers by Jurgoński et al. (2013), Jurikova et al. (2012a), Becker and Szakiel (2019), and Rupasinghe et al. (2018). With a circumpolar geographic distribution, predominantly thrives in the boreal and arctic forest zones of Eurasia, this plant is a mesophytic perennial fruit-bearing shrub that originated from Siberia and northeastern Asia and boasts remarkable characteristics as an emerging fruit crop, such as exceptional resilience to harsh winters, flower resistance against severe frosts, and early-season phenology. Blue honeysuckle primarily thrives in boreal and temperate coniferous woodlands, as well as shrublands, fens, and marshes, often growing as undergrowth within forest ecosystems (Mucina, 1997). Like many circumpolar species within the *Lonicera* genus (Rudenberg and Green, 1969), it tends to inhabit river valleys, boreal forests, and forest patches reaching into the tundra in the north, and extending up to mountain timberlines at higher elevations (Skvortsov, 1986). Conversely, in its southernmost regions, it is limited to the upper part of the forest belt and, under protective conditions, may extend into the lower parts of the sub-alpine and alpine zones (Rudenberg and Green, 1969). The northern boundaries of its native distribution are constrained by insufficient summer warmth, severe frosts, and nutrient-poor soils, while heat and drought define its southernmost limits (Sheyko, 2009).

The initial documentation of this plant traces back to the 17th century, while initial endeavors towards its cultivation occurred in Russia in the early 20th century. Ivan Vladimirovich Michurin was a Russian practitioner of selection to produce new types of crop plants, he started his research in pomology and selection and is one of the pioneers that began the study of blue honeysuckles in 1909 in the Tambov region of Russia and recommended to use this plant in orchards. From around 1950, Russian efforts intensified to breed cultivars aimed at maximizing yield, enhancing fruit size and sweetness, and facilitating mechanical harvesting through balanced fruit ripening. Comparable initiatives in various European countries, including Poland, Czech Republic, Lithuania, Finland, and Slovakia, commenced only towards the end of the 20th century (Celli et al., 2014; EFSA, 2018; Becker and Szakiel, 2019).

Presently, the commonly cultivated Canadian cultivars are derived from the cross-breeding of *L. caerulea* var. *kamtschatica* with the Canadian variety *L. kamtschatica* var. *villosa* and the Japanese (Hokkaido) variety *L. caerulea* var. *emphyllocalyx* (Thompson and Barney, 2007). Conversely, Polish varieties issued from the hybridization of *L. caerulea* var. *kamtschatica* with *L. caerulea* var. *edulis* (Becker and Szakiel, 2019). These particular species yield delectable, fragrant, sweet-and-tart fruits reminiscent of highbush blueberries or bilberries.

In Poland, the assortment include 'Wojtek', 'Jolanta', 'Atut', 'Duet', 'Brazowa', 'Czarna', and 'Warszawa' (Becker and Szakiel, 2019; Kaczmarska et al., 2015; Ochmian et al., 2012; Ochmian et al., 2008). Meanwhile, the most popular Canadian varieties are 'Blue Belle', 'Blue Bird', 'Blue Moon', 'Blue Velvet' 'Tundra', 'Aurora', 'Borealis', 'Indigo Gem' and 'Honeybee' (Becker and Szakiel, 2019; Rupasinghe et al., 2018; Rupasinghe et al., 2012). The fully developed shrub displays a dense, upright shape. These shrubs can grow up to 2 meters tall and spread to a width of 1.5-2 meters (Figure 1).

Figure 1. *L. caerulea* var. 'Zojka' in the experimental field of the Faculty of Horticulture Bucharest

The bush blossoms concurrently with leaf development. Their flowers are soft yellow, rich in nectar, with a delicate, pleasing scent.

Research has demonstrated significant variations in flowering time, sometimes exceeding two weeks, among identical varieties in different years. In Canada, blue honeysuckle typically starts flowering in early May, whereas in Poland, it usually blooms by late April (Gawroński et al., 2014). However, the timing of flowering is greatly influenced by climatic factors, especially temperature (Figure 2).

Figure 2. *L. caerulea* var. 'Blue velvet' in the experimental field of the Faculty of Horticulture Bucharest

Another significant factor is the variety itself, flowering duration can range from 7 to 15 days depending on the specific variety (Dawson, 2017). Given that blue honeysuckle is not self-pollinating, it relies on the presence of a different variety flowering simultaneously nearby for cross-pollination to take place. While a solitary blue honeysuckle shrub can still produce fruit, the yield is typically less abundant (Frier et al., 2016).

Blue honeysuckle typically begins bearing fruit in the second year after planting, with the full harvest potential, ranging from 3 to 5 kg, achievable 8 to 15 years post-planting (Dawson, 2017). The berries are characterized by their fleshy, elongated shape, navy blue coloration, and are adorned with a waxy, blue coating.

MATERIALS AND METHODS

The research was conducted in the experimental field of the Faculty of Horticulture - University of Agronomic Sciences and Veterinary Medicine of Bucharest (Figure 3) and Research Institute for Fruit Growing Pitești - Mărăcineni. The plant material consisted of 16 varieties of *Lonicera caerulea* from a young orchard established in micro-tunnels and 3 varieties of *Lonicera kamtschatica* planted in open field in the spring - autumn of 2023 at the distances of 3.0 × 1.0 m. The varieties of *Lonicera caerulea* included in this study were as follow: 'Zojka', 'Wojtek', 'Ruth', 'Rebecca', 'Larisa', '*L. kamtschatica*', 'Atut', 'Jolanta', 'Eisher', 'Indigi jam', 'Blue velvet', 'Blue pacific', 'Blue moon', 'Blue forest', Aurora', 'Borealis' and 'Kami', 'SI-15' 'SI-22' (from ICDP Piteşti - Mărăcineni).

Figure 3. Experimental field with the *Lonicera caerulea* varieties at USAMV Bucharest

The habit and vigour of the plants were evaluated by measuring the height, diameter, number of stems/plants, number of annual growths/plants, Σ of the annual growths.
The fructification capacity was assessed by the number of inflorescence and number of flowers and fruits per plant.
The mature leaves were analysed using the WinFolia 2022 software, measuring the leaf blade length and width, length of the petiole, and the basal and apical angles of the blade.
For morphological and micromorphological analyses, flowers and leaves were collected in April 2024 and examined fresh under a binocular and microscope. The biological material was studied using an Optica microscope and photographed with a Motorola digital camera in the Botany-Morphology and Plant Anatomy laboratory at USAMV Bucharest. Additionally, photographs were taken with a Motorola camera and a Leica S8AP0 binocular at various magnifications. Flowers were analysed at full bloom.
Diameter of the open flower, length of the petals and pistil, with of the petal and the number of stamens were measured or counted for all varieties.
Fruits were sampled at maturity, calculating their weight, widths, length, firmness, total sugars and total acidity.
From each variety it was collected 20 fruits for the morphological measurements done using a digital caliper and the weight was calculated as an average of 10 fruits/variety using the electronic balance Partner PS 1200 R2.
The total sugar was measured using the digital refractometer Milwaukee MA871.
For the pH and fruit's acidity, it was used a titrator device SI Analytics Titroline 5000 and for the firmness the Penetrometer 53205SP. Dry matter content was also recorded using oven Memmert GmbH.
Data retrieved were subjected to statistical analysis using one-way analysis of variance and the multiple range test Duncan by SPSS system.

RESULTS AND DISCUSSIONS

In the Spring of 2024, after the vegetation start and in the timeline with the occurency of the flowers at the variety level the samples were subsequntely collected and analyzed in the lab according to the methodology previously described.

Flowers
The size of the flowers varied between 1.2-2cm, are pale yellow, hermaphrodite, with a differentiated floral cover in the calyx and corolla, the sepals are small, the petals are 5 united at the base, 5 stamens arranged in alternation with the sepals (Figures 4, 5).

Figure 4. Flowers of *Lonicera caerulea*

The anthers are exserted, dorsofixed with a longitudinal opening. The style exceeds the corolla, nectaries may be present in the corolla tube.
The flowers are arranged in inflorescences. The inflorescences can have 2, 3 or sometimes 4 flowers.

Figure 5. Dorsifixed stamens with longitudinal opening

The corolla tube presents numerous secretory hairs and tectors (Figures 6, 7, 8).

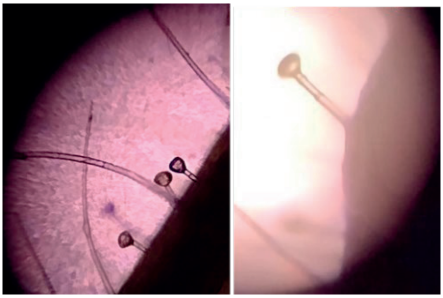


Figure 6. The corolla tube of *Lonicera caerulea*

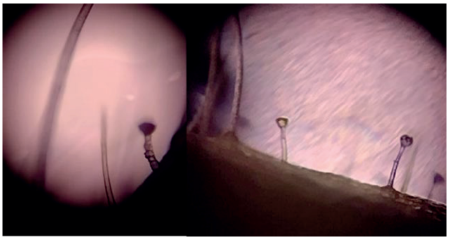


Figure 7. Secretory and tectors hairs in the corolla tube of *Lonicera caerulea*

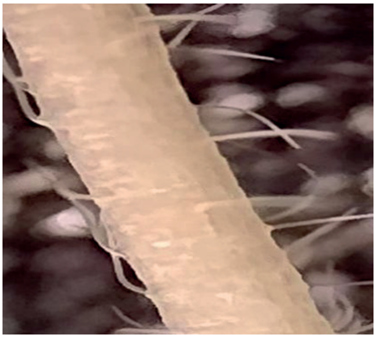


Figure 8. Upper epidermis with tector and secretory hairs

Leaves

The measured leaf parameters are presented in Table 1. The leaves have more tector and secretory hairs on the upper epidermis than on the lower one (Figures 9 and 10).

Figure 9. Lower epidermis with tector and secretory hairs

Figure 10. Superior epidermis with tector and secretory hairs

Fruits

Fruits characteristics are summarized in Table 2. The weight varied from 0.77 g at 'Eisher' to 1.69 g at 'Blue Pacific' with an average of 1.13 g.
The shape and color of the varieties are emphasized in the Figure 11, with distinctive redish tones at 'Rebeca' variety and darker blue at 'Blue Moon'. Smallest fruits were observed at the both selections of Pitesti.

Tabel 1. Leaf parameters of *Lonicera caerulea* varieties

Variety	Leaf Area (mm^2)	Perimeter (mm)	Vert Length (mm)	Horiz Width (mm)	Avg Horiz Width (mm)	Aspect Ratio (W/L)	Form Coefficient	Blade Length (mm)	Max Perp Width (mm)	Posi Max Perp Width (mm)	Perp Width 1 (mm)	Perp Width 2 (mm)	Lobe Angle 1	Lobe Angle2	Petiole Length (cm)	Petiole Area (cm^2)
Zojka	162.78	164.39	65.21	35.91	24.99	0.55	0,75	64.10	35.63	31.73	35.16	15.68	42.60	41.7	0.24	0.02
Wojtek	93.38	99.84	58.45	35.49	24.21	0.61	10.22	37.30	35.30	8.81	31.04	11.17	70.00	58.20	0.26	0.04
Ruth	83.57	116,67	47.15	24.70	17.32	0.52	0,75	46.74	24.39	23.51	24.21	12.26	41.00	38.38	0.10	0.01
Rebecca	121.71	143.77	58.62	30.56	20.67	0.52	0,74	58.11	30.12	28.58	29.85	12.97	32.2	38.20	0.16	0.02
Larisa	108.12	130.89	51.00	31.21	21.26	0.62	0.79	49.91	30.97	24.71	30.82	14.12	38.63	43.82	0.20	0.02
L. kamtschatica	115.42	139.44	56.28	29.88	20.43	0.53	0.74	55.51	29.63	23.86	29.07	11.46	43.82	41.64	0.16	0.03
Atut	153.70	153.77	57.52	37.13	26.55	0.65	0.81	58.13	36.37	28.207	36.15	16.98	46.64	45.27	0.23	0.03
Jolanta	122.68	140.29	57.38	31.83	21.34	0.56	0.78	57.23	31.03	27.43	30.76	13.63	31.09	38.73	1.07	0.04
Eisher	57.11	100.00	44.29	19.06	13.01	0.43	0.71	42.68	18.66	20.39	18.18	1.96	36.4	32.40	0.13	0.08
Indigi jam	134.85	147.39	62.43	32.20	21.71	0.52	0.78	60.05	31.98	27.85	31.66	12.84	45.09	40.18	0.36	0.07
Blue velvet	113.78	132.62	53.03	30.81	21.25	0.58	0.80	52.32	30.47	26.13	30.11	14.95	43.00	40.89	0.14	0.01
Blue pacific	151.26	156.31	64.02	33.59	23.24	0.53	0.76	62.72	33.30	29.10	33.02	15.67	38.2	39.30	0.19	0.03
Blue moon	153.34	162.30	67.34	34.39	22.78	0.51	0.72	65.88	34.07	30.94	33.78	14.62	23.5	38.40	0.24	0.03
Blue forest	121.70	140.84	57.45	31.32	21.12	0.55	0.77	56.27	30.95	27.206	30.59	12.34	38.5	40.3	0.21	0.02
Aurora	108.25	144.85	61.69	25.21	17.38	0.41	0.64	60.70	24.90	29.07	24.25	11.52	35.09	32.67	0.17	0.01
Borealis	81.23	126.54	53.44	22.99	15.13	0.43	0.63	52.83	22.67	24.67	22.19	2.07	31.1	33.50	0.16	0.01
Kami	84.00	121.15	49.66	23.97	16.86	0.48	0.71	49.65	23.61	23.85	23.20	6,44	45.8	36.90	0.06	0.005
SI-15	69.85	113.22	47.27	21.47	14.77	0.46	0.68	46.96	21.22	22.21	20.98	1.84	41.3	35.10	0.08	0.006
SI-22	63.13	107.99	45.00	20.45	13.97	0.46	0.68	44.99	20.06	23.55	19.622	2.20	40	34.00	0.10	0.01

Table 2. Fruit parameters of *Lonicera caerulea* varieties

Varieties	Weight (g)	Width 1 (mm)	Width 2 (mm)	Length (mm)
Zojka	1.04^{efg}	8.79^{g}	9.48^{def}	22.26^{c}
Wojtek	1.10^{def}	10.52^{def}	8.97^{efg}	23.78^{ab}
Ruth	0.92^{fgh}	9.80^{efg}	8.82^{efg}	15.89^{h}
Rebecca	1.43^{ab}	11.22^{bcd}	9.71^{cde}	20.58^{cd}
Larisa	0.88^{gh}	10.47^{def}	8.50^{fgh}	20.43^{cd}
L. kamtschatica	1.19^{cde}	12.30^{ab}	10.31^{abc}	17.00^{efg}
Atut	1.35^{abc}	10.89^{cde}	10.06^{bcd}	23.60^{ab}
Jolanta	1.12^{def}	9.39^{fg}	8.23^{gh}	22.58^{b}
Eisher	0.77^{hi}	8.85^{g}	7.84^{h}	19.72^{d}
Indigi jam	1.31^{abcd}	12.56^{a}	10.54^{ab}	19.47^{d}

Varieties	Weight (g)	Width 1 (mm)	Width 2 (mm)	Length (mm)
Blue velvet	1.06^{efg}	10.28^{def}	8.96^{efg}	18.43^{def}
Blue pacific	1.69^{a}	12.46^{ab}	10.74^{a}	22.73^{b}
Blue moon	1.16^{cde}	11.79^{abc}	9.68^{cde}	18.44^{def}
Blue forest	1.02^{efg}	10.30^{def}	9.12^{defg}	18.91^{de}
Aurora	1.06^{efg}	10.05^{def}	8.34^{gh}	23.30^{ab}
Borealis	1.04^{efg}	10.64^{cde}	9.09^{defg}	16.39^{gh}
Kami	0.68^{i}	3.43^{h}	3.10^{i}	2.46^{a}
SL-15-17	0.44^{j}	2.87^{hi}	2.60^{i}	1.62^{h}
SL-22-17	0.50^{j}	2.10^{j}	2.23^{i}	1.94^{d}

*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan’s multiple range test; p≤ 0.05.

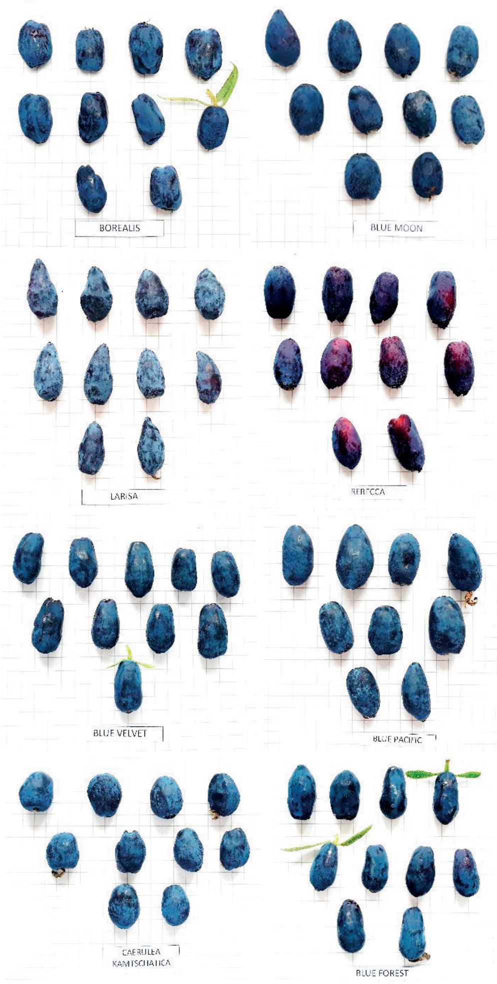


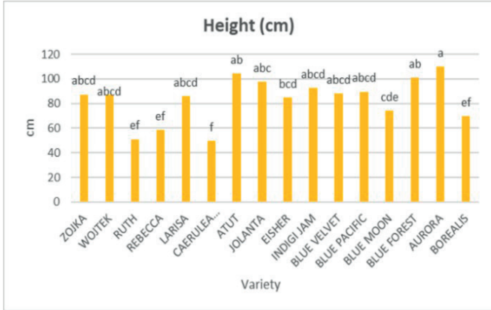
Figure 11. Shape and color of some *Lonicera* varieties at the harvest time

Concerning the plant behavior in terms of vigor and biometric particularities after the first vegetation period, we observed that differences at the variety level were consistent and statistically assured.

For instance, the tallest plants (Figure 12) were 'Aurora' variety that overpassed the other varieties together with 'Atut' which grew higher than 100 cm.

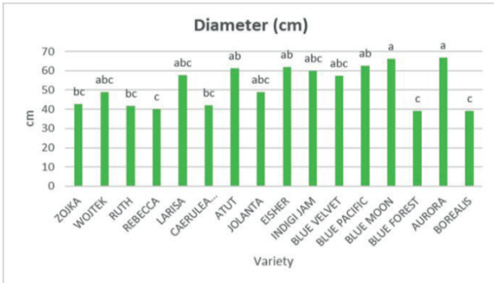
An homogenous group was remarked between 85 to 100 cm and the smallest ones seized at 'Ruth', 'Rebecca' and '*L. kamtschatica*'.

The plant shape is also very different from one variety to another, with the tendency of bigger diameter of the plants at the same variety with higher stems (Figure 13).



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$.

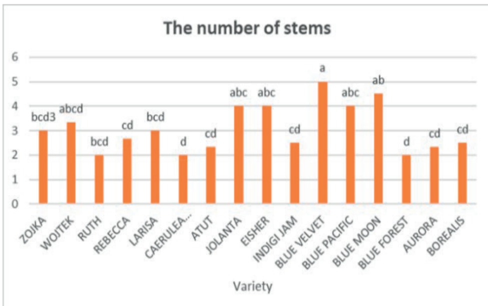
Figure 12. The differences in vigour at the level of *Lonicera* varieties



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$.

Figure 13. The plant diameter at the *Lonicera* varieties

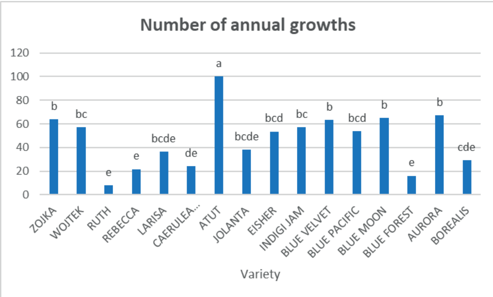
An interesting observation is related to the number of stems/plant (Figure 14.) that are not anymore correlated with the hight of the plants, in this case, 'Aurora' and 'Atut' registered less than 3 stems/plant while the group of 85-100 cm height encountered more that 4 stems/plant.



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$.

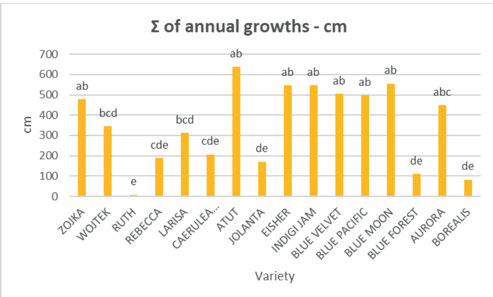
Figure 14. The number of stems/plant at the *Lonicera* varieties

The number of annual growths (Figure 15) indicate 'Ruth' as a very weak vigour close by 'Rebecca' and 'Blue forest'.
A more active plants were remarked at 'Atut' as well as the sum of the annual growths (Figure 16).



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; p≤ 0.05.

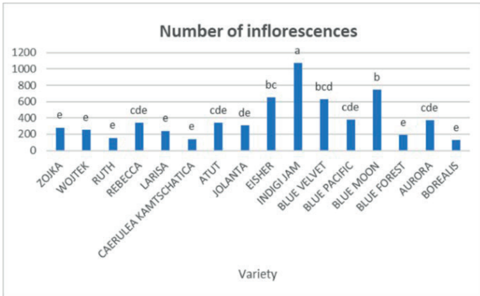
Figure 15. The number of annual growths/plant at *Lonicera* varieties



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; p≤ 0.05.

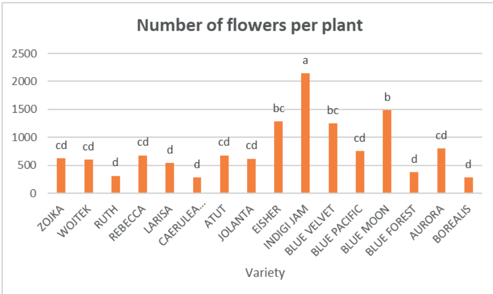
Figure 16. The total annual growths/plant at *Lonicera* varieties

In the second year after planting, all varieties of *Lonicera* sp set up fruits. The fructification parameters are indicating the variety 'Indigi jam' as a very fertile one considering the higher values of the number of inflorescences/plant (Figure 17) and the total number of flowers/plant (Figure 18).
Fruits of *Lonicera caerulea* are very soft and therefore with a short storability and shelf life capacity.
The quality of the fruits starts from the size and continue with the most use physio-chemical characteristics that define the overall value of the variety.



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; p≤ 0.05.

Figure 17. The number of inflorescences formed/plant at *Lonicera* varieties



*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; p≤ 0.05.

Figure 18. The number of flowers/plant at *Lonicera* varieties

In the Table 3, data showes that the firmest fruits were from 'Zojka', 'Wojtek' and *L. kamtschatica* where the values are over the threshold of 5 kgf/cm^2.
Sweeter fruits were harvested from *L. kamtschatica* (13.44^{0}Brix), 'Indigi jam' with 14.05^{0}Brix and both selection from Pitesti while 'Jolanta' accumulated only 10.09^{0}Brix. Far away from the other varieties is 'Ruth' which riched a percent of 23.19 dry matter. 'Eisher' and the same 'Indigi jam' proved to have the highest contents of dried matter as well as *L. kamtschatica* which had more than 15% of dry substance. pH values varied between 2 and 3 with a higher values at the Pitesti selections, close with the values reported by Gorzelany J et all, 2023. In their experiment, juice pH ranged between 3.7-3.32 at *L. kamtschatica* and 3.13-3.52 at *L. emphyllocalyx*. Gerbrand et al. (2020, found out a pH of *Lonicera caerulea* varying from 2.42 to 3.10.

Table 3. Fruit properties of *Lonicera caerulea* varieties at the first pick

Variety	Firmness (kgf/cm^2)	Total sugars (°Brix)	Dry matter content (%)	pH
ZOJKA	5.82^{c}	11.27^{ef}	12.47^{i}	2.77^{fgh}
WOJTEK	5.32^{cd}	11.33^{ef}	12.16^{ij}	2.64^{ijk}
RUTH	2.81^{ef}	13.2^{abcde}	23.19^{a}	2.78^{fg}
REBECCA	2.87^{ef}	11.28^{ef}	13.93^{f}	2.71^{ghi}
LARISA	3.41^{def}	12.57^{cde}	13.08^{h}	2.61^{jk}
KAMTSCHATICA	5.73^{c}	13.44^{abc}	15.77^{ab}	2.73^{ghi}
ATUT	2.53^{f}	12.99^{bcde}	13.78^{g}	2.71^{ghi}
JOLANTA	4.22^{cdef}	10.09^{f}	11.95^{j}	2.61^{jk}
EISHER	3.01^{ef}	12.73^{bcde}	15.06^{cd}	2.77^{fgh}
INDIGI JAM	4.98^{cde}	14.05^{abc}	15.48^{bc}	2.76^{fgh}
BLUE VELVET	4.15^{cdef}	11.51^{def}	11.98^{j}	2.56^{k}
BLUE PACIFIC	3.73^{cdef}	12.34^{cde}	11.94^{j}	2.8^{fg}
BLUE MOON	3.86^{cdef}	11.36^{ef}	13.57^{fg}	2.83^{de}
BLUE FOREST	3.57^{def}	11.3^{ef}	12.95^{h}	2.81^{ef}
AURORA	4.12^{cdef}	12.56^{cde}	14.56^{e}	2.96^{gh}
BOREALIS	3.58^{def}	11.58^{def}	13.11^{h}	2.76^{ghi}
Kami	1.19	13.33^{abcd}	13.33^{gh}	3.08^{bc}
SL-15-17	1.24	14.43^{ab}	14.43^{e}	3.42^{ab}
SL-22-17	1.49	14.86^{a}	14.86^{cd}	3.81^{a}

*Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$.

CONCLUSIONS

The variability in traits for all varieties of *Lonicera* sp was more expressed for vegetative characteristics of plant and fruit size and less in case of pH, total sugar content and dry substance.

Morphological traits for all 19 varieties of honeysuckle indicated a large variability in terms of leaf and flower botanic characteristics.

In our growing condition, the most vigorous variety of *Lonicera* sp was 'Aurora' closely followed by 'Atut'.

'Blue velvet' presented the most spread growth among the varieties of *Lonicera* sp.

'Indigi jam' performed better in terms of number of flowers and fruits/plant and also for the total sugar content.

The biggest fruits were harvested from 'Blue Pacific' and the highest content of dry substance accumulated in the fruit was recorded at 'Ruth' variety.

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NON-DESTRUCTIVE ASSESSMENT OF STRAWBERRY FRUIT QUALITY

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Abstract

Conventional methods for fruit analysis are often destructive, time-consuming, and labor-intensive. Rapidly and non-destructively detecting fruit quality is an important topic in fruit agriculture. The aim of the research is a non-destructive assessment of the quality of strawberry fruits of three varieties grown in a greenhouse. A total 90 number of fruits were collected, 30 of each variety. The diffuse reflectance spectral data of all fruits in the region 900 to 1700 nm were obtained using NIRQuest (Ocean Optics, Inc.). The soluble solids content (°Brix), ascorbic acid, and texture parameters (rupture force, yield force, modulus of fresh elasticity, and deformation work) were measured on each fruit. PLS regression was used to create models for the determination of tested parameters. Good PLS equations were obtained for soluble solids content and ascorbic acid content. The parameter RPD = SD/SECV, which is used to evaluate the accuracy of the determination, has values of 3.06 for SCC and 4.57 for ascorbic acid content. The accuracy of defining textural parameters was excellent. For each of them, the correlation coefficient R_{cv} was 0.99 and the determination errors were small. No difference was observed in the accuracy of the determination of tested parameters depending on the strawberry varieties.

***Key words**: strawberry, soluble solids content, ascorbic acids, texture, NIR spectroscopy, PLS Regression.*

INTRODUCTION

Strawberries are one of the popular high-value fruits with numerous nutrients and health benefits. They are a rich source of vitamins, polyphenols dietary fiber, and minerals (Giampieri et al., 2012). Recent clinical studies have confirmed that strawberries can reduce the serum levels of the branched-chain amino acids valine and leucine as well as significant increases in untargeted metabolites, including serum phosphate, benzoic acid, and hydroxyphenyl propionic acid (Basu et al., 2023). The authors concluded that dietary supplementation of strawberries significantly improves the serum metabolic profiles of cardiometabolic health of adults.
Conventionally, strawberry quality has been evaluated with criteria such as color, texture, chemical constituents, etc., by fruit experts or researchers (Rico et al., 2007; Temocico et al., 2019). These methods are often destructive, time-consuming, and labor-intensive. Also, destructive methods cannot be applied to the entire batch of a product, but only to a certain number of fruits. Nowadays people's requirements in terms of food quality are increasing. Non-destructive testing methods and chemical-free analytical procedures are needed for farmers, in the supply chain of fresh strawberries or the process industry.
In recent years, with the development of near-infrared (NIR) spectroscopy and advanced optical imaging techniques such as multispectral and hyperspectral imaging in visible and NIR regions, a rapid increase use of these instruments can be seen in assessing the chemical composition and quality of fruits in a non-destructive way (Alander et al., 2013; Chandrasekaran et al., 2019; Aline et al, 2023; Guo et al., 2024). The development of spectrometers and the current availability of

portable devices allow analysis not only in the laboratory but also in the field, which is of particular importance for determining the maturity and quality of fruits and the most suitable moment for harvesting them.

Recent studies, related to the assessment of the sensory and nutritional quality of strawberries using visible and near-infrared spectroscopy have been published. The most tested parameters are soluble solid content, acidity, and firmness. Shao and He, 2008 reported a successful prediction model for acidity in strawberries based on wavelet transform combined with partial least squares (PLS) with a correlation coefficient of 0.856 and root mean square error of prediction (RMSEP) of 0.026. Sánchez et al., 2012 using a MEMS handheld instrument analyzed five commercial cultivars of strawberry. The authors reported the feasibility of NIRS for the prediction of firmness, soluble solid content, and titratable acidity. Amodio et al., 2017 investigated the potentiality of NIR spectroscopy to predict the internal quality attributes of organic and conventionally grown strawberries. The authors reported good prediction models for soluble solid content, pH, and total acidity. The other parameters - ascorbic acid and phenols, were predicted with much lower accuracy. Mancini et al., 2020 investigated the application of NIR spectroscopy for the prediction of soluble solid content, titratable acidity, firmness, and color of fresh strawberries. Good PLS models were obtained only for firmness and total soluble solids parameters. Another study of the same group (Mancini et al., 2023) proposes partial least squares discriminant analysis to classify four selected strawberry genotypes according to the quality parameters of soluble solids, vitamin C, anthocyanins, and phenolic acids contents. Saad et al., 2022 evaluated the potential of a hand-held Vis-NIR spectrometer to classify the maturity stage and to predict the quality attributes of strawberries. The developed models for the determination of colour parameters, total soluble solids, titratable acidity, and total polyphenol content had a coefficient of determination bigger than 0.9.

Hyperspectral imaging in the NIR range has been found useful for nondestructive estimation of the quality of strawberries. Tallada et al., 2006 reported that NIR hyperspectral images (650 to 1000 nm) and three-wavelength model (685, 865, and 985 nm) could predict firmness in strawberries. Hyperspectral imaging in the visible and near-infrared (400–1000 nm) regions was tested for nondestructive determination of moisture content, total soluble solids (considered a very important parameter in evaluation of the quality), and acidity in strawberries (ElMasry et al., 2007; Chen et al., 2018). The correlation coefficients bigger than 0.80 were reported for the prediction of tested parameters. A higher classification accuracy of 89.61% was achieved for classifying strawberries based on the ripeness stage. A hyperspectral imaging system covering two spectral ranges (380-1030 nm and 874-1734 nm) was applied by Zhang et al., 2016 to evaluate strawberry ripeness. The obtained classification accuracy was over 85%. An approach to visualize the spatial distribution of sugar content in white strawberry fruit flesh using near-infrared hyperspectral imaging in the region 913-2166 nm was developed by Seki et al., 2023.

The main objective of this study is to evaluate the capabilities of NIR spectroscopy for the nondestructive determination of quality and textile parameters of fresh strawberries from three varieties grown in a greenhouse.

MATERIALS AND METHODS

Strawberry samples

Strawberries belonging to three commercial cultivars - Asia, Alba, and Clery were used in this study (Figure 1). The strawberries were grown in a greenhouse of Trakia University academic technological complex, under irrigated conditions with drip irrigation. A total 90 number of fruits were collected, 30 of each variety. The Alba variety is created by crossing two varieties Albion and Cal. 97.85-6 from the Italian company New Fruits. The variety is characterized by conical, very uniform, bright red, and shiny fruits. The quality of the fruit is good and very stable. Variety Asia is an industrial variety of large and juicy fruits, first grown in Italy in 2005. Medium early variety that ripens 5 to 6 days later than Alba. The fruits are attractive, large, conical, very uniform, and extremely bright red.

a)

b)

c)

Figure 1. Investigated strawberry cultivars: Alba (a), Asia (b), and Clery (c)

The variety is characterized by high sugar content and the balanced acid ratio, the taste is delicious. The fruits of Clery variety have a regular conical shape, uniform. The color of the berries is dark red saturated with shine. The taste is sweet with a characteristic sourness.
In order to determine the quality parameters, the fruits were harvested at different maturity stages (half-ripe and ripe stage) to enlarge the interval of variation of tested parameters in the calibration models.

Measurement of quality parameters

Analysis of the mechanical characters and chemical compounds of the fruits was performed at the Laboratory for Vegetable Quality Control of Maritsa Vegetable Crops Research Institute - Plovdiv. Textural parameters were studied on the day of the harvest. The laboratory tests were conducted by TA.XT.Plus Texture analyser (Stable Micro Systems, UK) equipped with a Heavy Duty Platform (HDP/90) with holed plate and a 2 mm diameter stainless steel puncture probe (SMS P/2). The instrument was set at a test speed of 2 mm s^{-1} and a travel distance of 10 mm. The analysis was performed on individual fruits of investigated cultivars. The measurement was done in the equatorial part of the longitudinal slices. The force-deformation curves were analysed for yield force (1^{st} force maximum), modulus of flesh elasticity (slope up to 2^{nd} maximum), deformation work (area under the curve up to 2^{nd} maximum), and rupture force (force maximum) (Figure 2).

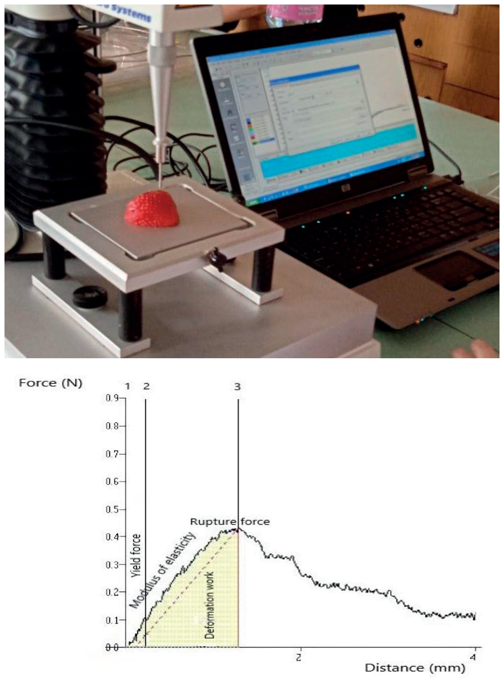

Figure 2. Stable Micro Systems TA.XT. Texture Analyser and typical force-distance curve of the fresh strawberries

Each strawberry fruit was analysed for soluble solids content (SSC) using a digital refractometer and ascorbic acid by Tilman's reaction with 2, 6-dichlorophenolindophenol (Genadiev et al. 1969).

Spectral measurements

NIRQuest 512 (Ocean Optics, Inc.) spectrometer in the range 900-1700 nm and optical resolution 3.1 nm. Spectral acquisition of tested 90 strawberry fruits was made using a reflection fiber-optics probe. The instrument was set to average 20 scans for one spectrum. The fruits were measured at three positions along its equator approximately spin 120 degrees.

The spectral data processing was performed with Pirouette 4.5 software (Infometrix, Inc.). PLS models were used for development models for quantitative determination of tested parameters with cross-validation. The optimum number of PLS factors in each model was defined to be the one that corresponded to the lowest standard error of cross-validation (SECV). Different mathematical treatments were evaluated - smoothing, multiple scatter correction, first and second derivative.

RESULTS AND DISCUSSIONS

Differences in measured parameters between strawberry varieties were found. The mean value of SSC was higher for the Clery variety – 8.80 and lower for the Asia variety – 6.89. Ascorbic acid content was higher for fruits from the Alba variety. Among textural parameters, the main differences were found for the modulus of flesh elasticity.

The range, mean values, and standard deviation for the total group of samples from three strawberry varieties for each measured quality parameter are reported in Table 1. A relatively high range of measured parameters' values was observed because fruits from different varieties were included in the data set.

The average absorbance spectra of the three tested strawberry varieties are presented in Figure 3. The spectra of the three cultivars were very similar with small differences among them. The absorption maxima were observed around 930, 990, 1190, and 1410-1470 nm. The biggest maxima in the region 1410-1470 nm might be assigned mainly to O–H vibration from water and sugars. The absorption at wavelength regions around 930 and 1190 nm might be related to C–H vibrations, and at 980 nm to O–H vibrations (Workman &. Weyer, 2008). Similar spectral characteristics of strawberries were reported by Seki et al., 2023.

Table 1. Quality attributes of strawberry fruits.

Parameters	Range	Average	SD*
Soluble solids content, ⁰Brix	4.6-12.1	8.39	1.85
Ascorbic acid, mg %	51.60-81.27	65.45	7.99
Yield force. N	0.241-0.765	0.429	0.148
Rupture force, N	0.384-1.185	0.682	0.229
Modulus of flesh elasticity, $N.mm^{-1}$	0.017-1.045	0.351	0.225
Deformation work, N.mm	0.080-0.905	0.339	0.229

*SD – standard deviation

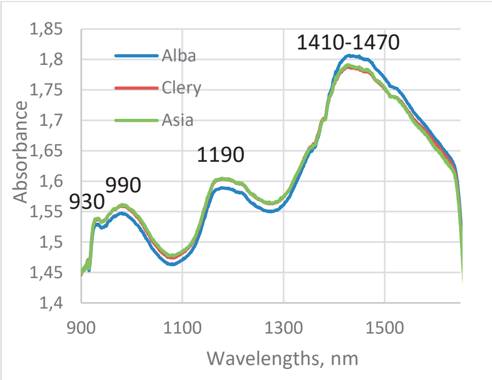

Figure 3. The average absorbance spectra of the tested strawberry varieties

Table 2 shows statistics for the best equations for the prediction of internal quality and textural parameters of the fruits based on the strawberries' near-infrared spectra. For most of the study parameters, slightly better results were obtained using the first derivative transformation of the spectral data. Mancini et al., 2020 also found the best PLS model for the determination of SSC using the first derivative transformation of strawberries' spectral data. The exception was only for the ascorbic acid content, which best equation was obtained by transforming the spectra as a second derivative. Between 5 and 8 PLS components were used in the regression equations.

Table 2. Calibration statistics for internal quality and textural parameters in strawberries using PLS regression

Parameter	SECV	R_{CV}	SEC	R_{Cal}
Soluble solids content, ^{0}Brix	0.605	0.93	0.569	0.94
Ascorbic acid, mg %	1.75	0.95	0.11	0.99
Yield force. N	0.007	0.99	0.004	0.99
Rupture force. N	0.016	0.99	0.008	0.99
Modulus of flesh elasticity. N.mm^{-1}	0.017	0.99	0.009	0.99
Deformation work. N.mm	0.004	0.99	0.002	0.99

SECV - standard error of cross-validation, R_{CV} - cross-validation correlation coefficient. SEC, Standard error of calibration, R_{cal} - coefficient of multiple correlation,

A graphical illustration of the accuracy of NIR spectroscopy prediction of tested parameters was presented in Figure 4 for ascorbic acid content, Figure 5 for soluble solids content, and Figure 6 for rupture force, respectively.

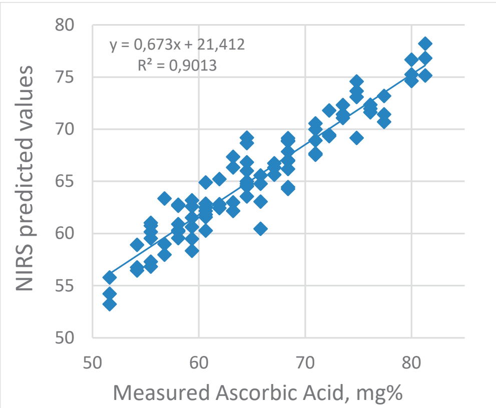

Figure 4. Regression plot of the Partial Least Squares Regression (PLS) model for predicting ascorbic acid content (cross-validation results)

Good PLS equations were obtained for SSC and ascorbic acid content. The parameter RPD=SD/SECV, which is used to evaluate the accuracy of the determination, has values of 3.06 for SCC and 4.57 for ascorbic acid content. RPD values greater than 3 indicate very good determination accuracy. No difference in the accuracy of determination of these parameters depending on the studied varieties was observed.
Similar results for the prediction of SSC and vitamin C were reported by Weng et al, 2000, based on VIR/NIR spectra (400-1000 nm) of strawberries at different ripeness stages.

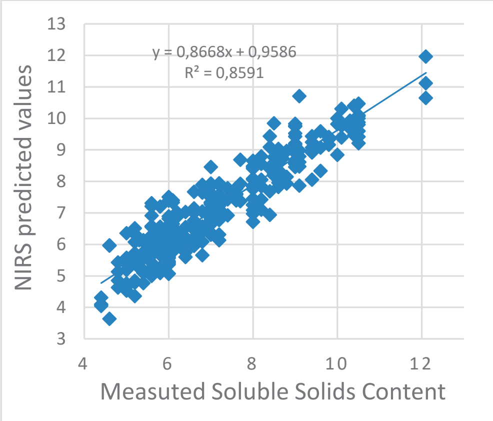

Figure 5. Regression plot of the Partial Least Squares Regression (PLS) model for predicting soluble solids content (cross-validation results)

The accuracy of defining textural parameters was excellent. For each of them, the correlation coefficient R_{cv} was 0.99 and the determination errors were small, as can be seen for example in Figure 6.

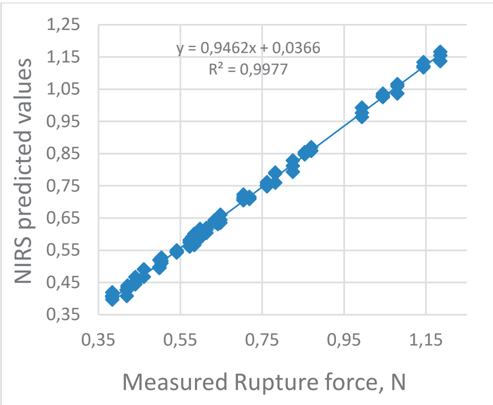

Figure 6. Regression plot of the Partial Least Squares Regression (PLS) model for the prediction of rupture force (cross-validation results)

CONCLUSIONS

The results show the potential of near-infrared spectroscopy for predicting the SSC, ascorbic acid and textural parameters of strawberries non-destructively from the fruit spectra.
Near Infrared Spectroscopy (NIRS) can be an alternative to the destructive methods for analysis of soluble solids content, ascorbic acid, and texture of strawberries.

Nowadays, some NIR spectrometers are portable and can be used outside the laboratory, in the field, to simultaneously evaluate the ideal harvest stage, and storage time, or to the development of fruit sorting systems to optimize their use.

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THE EFFECT OF SOME FOLIAR FERTILIZERS ON FRUIT QUALITY OF PEACH

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Abstract

The utilization of foliar fertilizers has become a fundamental management tool in intensive and super-intensive orchards. For this experiment, five peach genotypes ("DDD 67", "Maria Bianca", "Eureka", "HB 19-9" and "Tokinostate") were studied. Each genotype was treated with four different foliar fertilizers, two organic and two chemicals, during three separate growth phenophases. The genotypes were divided into five groups from which one group functioned as the experimental control and received no foliar treatment. In the case of the analyzed genotypes, the fertilizers, especially the biological ones, had significant positive influences on the internal characteristics of the fruits. Regarding the soluble solid content, in all genotypes, except "HB 19-9", better results were obtained in the case of biological fertilizers usage, especially the Cropmax fertilizer. The highest values of total polyphenol content, in all genotypes, except "Eureka", were also obtained in the case of biological fertilizers usage.

Key words*: foliar fertilizers; peach; total polyphenol content; soluble solid content.*

INTRODUCTION

Fertilizer application has become an essential management tool in intensive and super-intensive orchards, being one of the essential factors for ensuring high productivity and high quality harvests (Andreev et al., 2018; Barreto et al., 2020; Farias Barreto et al., 2022; Maatallah et al., 2024). The trend toward foliar fertilizers is increasing (Das & Mandal, 2015; Fernández & Eichert, 2009). Foliar fertilization strategies can increase nutrient use efficiency, and lessen their detrimental effects on the environment (Farahy et al., 2021; Niu et al., 2021; Otálora et al., 2018).

Persica vulgaris L. is considered an important species, that contributes to human nutrition, being the second-largest temperate fruit crop worldwide, after apple crop (Cantin et al., 2009; Manganaris et al., 2022).

Due to their high content of phenolic compounds and carotenoids, peaches have been associated with favorable effects on human health such as effects on the heart, chemoprevention, obesity, antidiabetic activity and neurodegenerative illnesses (Bento et al., 2022; Hussain et al., 2021; Mokrani et al., 2016; Noratto et al., 2014).

In the last period peach consumption had a negative trend. Studies on consumer preferences have linked the lower rates of peach consumption to overripe, tasteless, and/or immature fruit (Clareton, 2000; Crisosto, 2001; Iglesias, 2013; Manganaris et al., 2022).

Considering that fruit quality can only be obtained in the orchard, through optimal preharvest parameters (Minas et al., 2018) we aimed to study the effect of several foliar fertilizers in order to improve fruit quality in five peach genotypes.

MATERIALS AND METHODS

In order to carry out our research, five peach genotypes were studied: "DDD 67", "Maria Bianca", "Eureka", "HB 19-9" and "Tokinostate" (Figure 1), which were treated with four foliar fertilizers in three different growth periods. The first treatment was carried out in mid-May, the second treatment 3 weeks later, and the last treatment was carried out about 14 days before fruit ripening. Each variety was divided into four different groups, each group being treated in all phenophases with the same foliar fertilizer. Also, for each

group, controls were chosen, on which no fertilizer was applied.
The foliar products used were as follows: Cropmax (1.5 L/ha), Albit (100 mL/ha), Solfert (4 kg/ha) and Foliq N Universal (5 L/ha).
The experiment was carried out during the year 2022 at Pohalma Nursery in Lugoj county (45°42'22.1"N 21°51'36.1"E).
The trees were planted in 2015 in a 4 x 4 m planting layout and are all grafted on Oradea peach rootstock and trained in a “vase-shape".
Fruit analysis involved the determination of the soluble solid content (SSC), fruit moisture and antioxidant activity (total polyphenols content).
In order to analyze the specific parameters, 15 samples of fruit picked at the optimal ripening stage were taken from each group of the 5 genotypes.
The complex chemical determinations and analyses were carried out within the Research Platform of the University of Life Sciences “King Mihai I” from Timișoara, using specific methods in accordance with those reported in the literature (Altemimi et al., 2015; Cirilli et al., 2016; Di Vaio et al., 2008; Fauriel et al., 2007; Golisz et al., 2013; Kumar et al., 2010; Mihaylova et al., 2021; Minas et al., 2018; Tan et al., 2022).
The equipment used for analyses are: ATAGO PAL 3870 digital refractometer (soluble solid content); Binder FD 115 oven (water content); UV-VIS Analytic Jena Specord 205 spectrophotometer (total polyphenols content (TPC)).
Statistical calculations were performed using Microsoft Excel and SAS Studio SAS® Studio 3.8 software, with application of the One Way Anova test.

Figure 1. The studied varieties and genotypes of peaches

RESULTS AND DISCUSSIONS

The results determined for the examined parameters (soluble solid content, fruit moisture and total polyphenols content) are presented in Figures 2-4.

Soluble solid content

The fruits of the "DDD 67" genotype recorded values of the SSC between 9.4 °Brix (Foliq N) and 22.1 °Brix (Albit), with an experimental average of 15.51°Brix, with no significant differences recorded. The highest values (18.03 °Brix on average) were obtained with Cropmax fertilizer, and the lowest were obtained in the control (13.57 °Brix on average).
For the "Maria Bianca" variety the recorded values range between 14.4 °Brix (Solfert) and 18.9 °Brix (Cropmax fertilizer), with an experimental average of 17.03 °Brix, with no significant differences. The highest values (18.17 °Brix on average) were obtained with Cropmax fertilizer, and the lowest with Solfert fertilizer (15.63 °Brix on average).
In the "Eureka" variety, the SSC values were between 10.6 °Brix (Solfert fertilizer) and 17.2 °Brix (Foliq N fertilizer), with an experimental average of 13.73 °Brix, with significant positive differences for Cropmax fertilizer compared to Solfert fertilizer. The highest values (16.03 °Brix on average) were obtained with Cropmax fertilizer, and the lowest with Solfert fertilizer (11.04 °Brix on average).
The "HB 19-9" genotype recorded values between 18.4 °Brix (Foliq N) and 23.4 °Brix (Solfert fertilizer), with an experimental average of 21.02 °Brix, with no significant differences recorded. The highest values (22.37 °Brix on average) were obtained in the case of the control, being the only variety in which the control recorded better results compared to all the fertilizers used. The lowest values were obtained with Cropmax fertilizer (19.63 °Brix on average).
The fruits of the "Tokinostate" variety recorded values of the SSC between 12.4 °Brix (the control) and 18.8 °Brix (Cropmax fertilizer), with an experimental average of 15.73 °Brix, with significant positive differences for Albit fertilizer compared to the experience control ($p>0.0169$). The highest values (17.6 °Brix on average) were obtained with the Albit fertilizer, and the lowest were obtained with the control (13.6 °Brix on average).

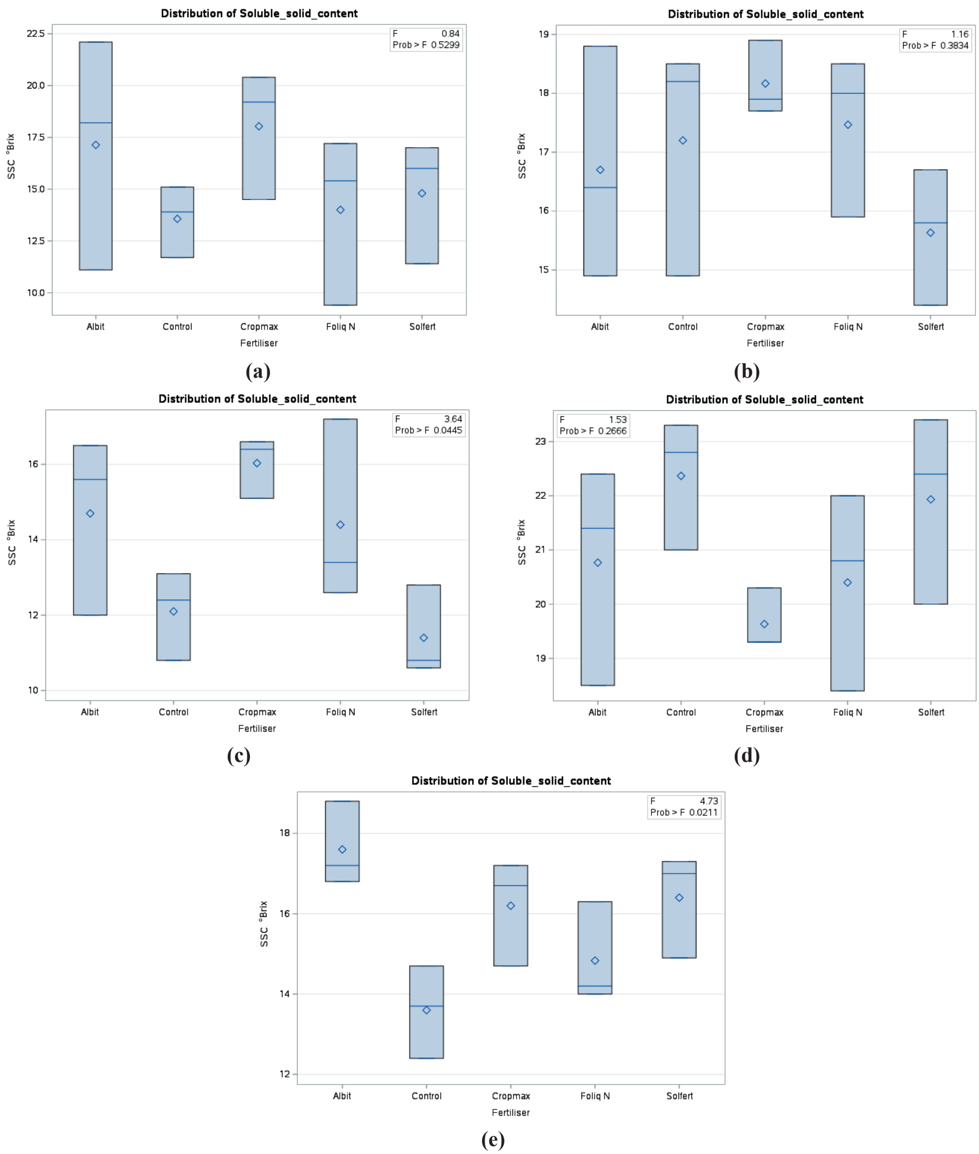

Figure 2. The influence of the fertilisers on the SSC: **(a)** "DDD 67" genotype; **(b)** "Maria Bianca" variety; **(c)** "Eureka" variety; **(d)** "HB 19-9" genotype; **(e)** "Tokinostate" variety

Fruit moisture content

The optimum moisture content for peaches varies between 80% and 90% (Bauman et al., 2005; Kumar et al., 2010; Lufu et al., 2020). Moisture content is a crucial factor in food quality, preservation and also in increasing resistance to deterioration (Nielsen, 2010).

As can be observed in Figure 3, the moisture content of the fruits varied depending on the genotype, but also on the fertilizer used, with significant differences being recorded in each group. The lowest moisture content values were recorded for the "HB 19-9" genotype (76.57-82.37%), and the highest values for the "Eureka" variety (85.21-90.27%).

On average, the highest humidity was recorded in the case of the controls (in two of the genotypes: "DDD 67", "Tokinostate") and in the case of the use of Solfert fertilizer, and the lowest values were recorded in the case of the use of Albit and Cropmax fertilizers.

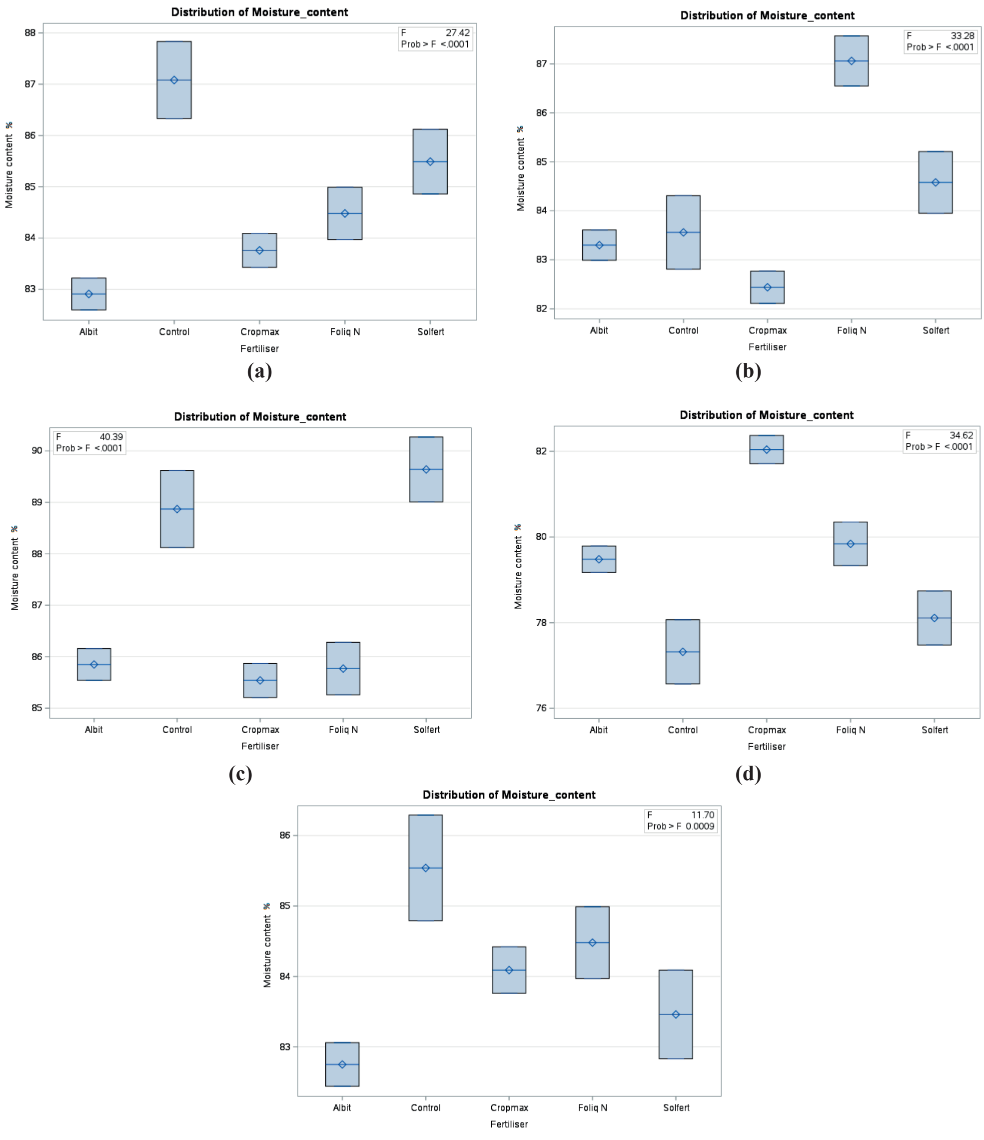

Figure 3. The influence of the fertilisers on the fruit moisture content: **(a)** "DDD 67" genotype; **(b)** "Maria Bianca" variety; **(c)** "Eureka" variety; **(d)** "HB 19-9" genotype; **(e)** "Tokinostate" variety

Total polyphenols content

Phenolic compounds are secondary metabolites widely found in fruits, mainly represented by flavonoids and phenolic acids. The increase in interest in these substances is largely due to their antioxidant potential and the relationship between their consumption and the prevention of certain diseases. The health benefits of these phytochemicals are closely related to regular consumption and their bioavailability. Studies have shown the importance of regular fruit consumption, especially for the prevention of diseases associated with oxidative stress (Andreotti et al., 2008; Di Lorenzo et al., 2021; Fraga et al., 2019; Haminiuk et al., 2012; Lima et al., 2014; Mushtaq & Wani, 2013; Vauzour et al., 2010).

Regarding the total polyphenol content of the analyzed genotypes (Figure 4), it varied depending on the genotype, but also on the fertilizer used, with significant differences being recorded in each group.

The lowest content in polyphenols (on average) was recorded in the "HB 19-9" genotype (429.14 mg/kg), and the highest in the "Maria Bianca" variety (1103.52 mg/kg).

The highest values (on average) were obtained with the following fertilizers:

- Albit: 1011.86 mg/kg for the "DDD 67" genotype and 1036.39 mg/kg for the "Tokinostate" variety;
- Cropmax: 1703.57 mg/kg for the "Maria Bianca" variety and 564.26 mg/kg for the "HB 19-9" genotype.

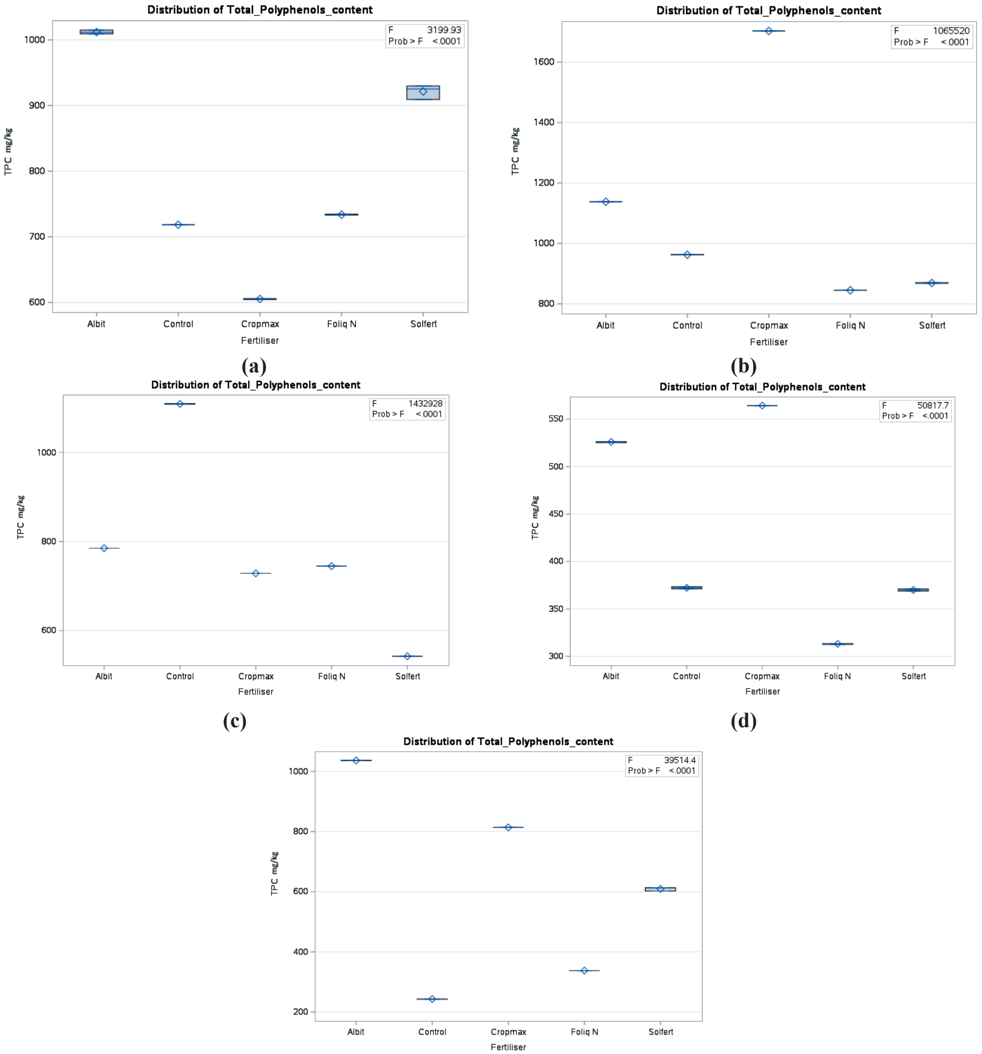

Figure 4. The influence of the fertilisers on the Total polyphenols content: **(a)** "DDD 67" genotype; **(b)** "Maria Bianca" variety; **(c)** "Eureka" variety; **(d)** "HB 19-9" genotype; **(e)** "Tokinostate" variety

CONCLUSIONS

The selection of the optimal fertiliser is influenced especially by the variety.

Regarding the soluble solid content, in four out of the five genotypes, better results were obtained in the case of the use of biological fertilizers (especially Cropmax fertilizer), only in the case of the "HB 19-9" genotype were obtained better results in the case of the control, being the only variety in which the control recorded better results compared to all the fertilizers used.

Regarding the fruit moisture, on average, the highest values were recorded in the case of the controls (in two of the genotypes: "DDD 67", "Tokinostate") and in the case of the use of Solfert fertilizer.

Regarding the total polyphenols content, the highest content in polyphenols (on average) was recorded when using fertilizers Albit and Cropmax.

In conclusion, in the case of the analyzed genotypes, the fertilizers, especially the biological ones, had significant positive influences on the internal characteristics of the fruits.

Further research is necessary, especially regarding the influence of the biological fertilisers.

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OBSERVATIONS ON ARTHROPODS EXISTING IN SOME ARONIA PLANTATIONS IN THE NE AREA OF ROMANIA

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Abstract

Black chokeberry (Aronia melanocarpa L.) is a fruit-bearing shrub that has come to the attention of growers due to the content of antioxidants and vitamins in the fruit. Being a perennial crop, it provides stable habitats for both useful and harmful entomofauna. The aim of the work was to evaluate the diversity of existing arthropods in an aronia plantation within the experimental plots of RSFG Iași. The experience was organized in four variants: V1- in which approved treatments for ecological agriculture were applied; V2- ecological treatments and irrigation, V3- in which approved conventional treatments, V4-conventional treatments and irrigation, each variant having three repetitions. Arthropod densities were sampled using Barber-type soil traps, in which the fixing solution was sodium chloride (NaCl) with a concentration of 25%. The harvestings were made periodically, from the beginning of May to the end of September 2022. The structure of the collected material varied according to the experimental variant. The arthropod fauna was made up of species that systematically fall into the following orders: Coleoptera, Hymenoptera, Heteroptera, etc.

***Key words**: arthropods, black chokeberry, treatments, useful entomofauna.*

INTRODUCTION

Black chokeberry (*Aronia melanocarpa* L.) is a shrub native from North America being one of the most cultivated species in recent years in Romania (Chiorean et al., 2023). This berry shrub is cultivated especially for the astringent fruits, which is very high in several healthful compounds, but are introduced in landscape design compositions or isolated plant, also fulfilling an ornamental role (Çelik et al., 2022, Ünal et al., 2023). In the studies and observations carried out over time, aronia is a plant with high ecological plasticity, having the ability to capitalize very well on a diversity of pedo-climatic conditions. This fact was highlighted by the reporting of this species in the spontaneous flora and considered an invasive species in some habitats (Vinogradova et al., 2018; Dinu et al., 2022).

Being a perennial crop, aronia makes use of the same land for a longer period of time, and the shrubby habitus can accommodate both useful and harmful fauna to the ecosystem. Known to be a shrub without too many pests, it is part of the Rosaceae family and can be attacked by specific diseases and pests: aphids, mites, bedbugs or cycads. The choice of host plants by the phytophagous insects depends on number of factors which have a significant influence or not on the physiology and behaviour of plant-eating insects but also on the chemism of plants defense (Piersanti et al., 2023).

Recently, one of first insect problems seen in the flowering time of aronia was with *Lytta* sp. and *Epicometis hirta* Poda (Tretiacova & Todiraș 2017) which consumes the inflorescences. Some studies refer to the *Acrobasis advenella* Zinck., whose larvae feed on inflorescences (Górska-Drabik et al., 2013). Thus, the damage caused by some pests may be increasing in these plantations and the application of pesticides that have increased toxicity have negative effects on the environment and may produce large imbalances on the ecosystems.

Therefore, modern control methods that involve ecological methods, but also natural methods that are based on the ability of the

biosphere to self- adjustment are being promoted a lot (Wackernagel & Galli, 2007).
The aim of this work was to evaluate and compare the diversity of existing arthropods in an aronia plantation under the four experimental variants. This will determine the subsequent choice of the most effective cultural methods in aronia plantations.

MATERIALS AND METHODS

This study was carried out in a chokeberry crop from the experimental plot of Research Station for Fruit Growing Iași. The site is located in the forest steppe of Moldova (47°20´N; 27°60´E, 165 m altitude) on the geomorphological unit characterized by a meso-relief where the predominant type of soil is cambic chernozem. The climate of this area is considered Dfb type according to the Köppen-Geiger classification (Belda et al., 2014) with an average annual temperature of 10.2°C and a significant rainfall of 562.6 mm, usually distributed throughout the year.
The experience was organized in four variants, with three replied, as follows:
V1 - in which approved treatments for ecological agriculture were applied;
V2 - ecological treatments and irrigation;
V3 - in which approved conventional treatments;
V4 - conventional treatments and irrigation.
In the V2 and V4 variants was used drip irrigation at different water regimes depending on the rainfall and the needs of the plants in different phenological stages. Thus, the soil water content in the depth of 0-50 cm was increased up to the capacity of the field by applying a watering rate of 6 litres/hour.
During the study period, the diversity of existing arthropods was evaluated within the experimental variants. Their collection was done with the help of Barber-type method and the samples were taken every 14 days between May and September. The trap consisted of 0.8 litre glass cups with an opening of 8.0 cm diameter. They were protected from the rain and other impurities with 10 × 10 cm metal roofs.
The soil traps were placed three on each variant at a distance of 10 meters. The containers were placed at ground level in which a fixing solution of sodium chloride (NaCl) with a concentration of 20% was added.
By placing the 12 traps, all categories of arthropods can be collected from the biotope, in order to later establish some ecological indicators such as abundance (A), constancy (C), dominance (D), the index of ecological significance (W).
Abundance (A) is the representation of a species in a certain ecosystem that is usually measured in the number of individuals captured per sample (Bonar et al., 2011). Based on this indicator, the other indicators are calculated (Vălean et al., 2018).
Constancy (C) is a structural indicator expressed by the continuity of the appearance of a species in the analyzed biotope. The higher value of this indicator, the better adapted the respective species is to the conditions encountered in the respective biotope (Battes, 2018).
Constancy was expressed according to the following formula:

$$\boldsymbol{C_A = \frac{n_p A}{N_p} \times 100}, \text{ where:}$$

$\boldsymbol{C_A}$ - the constancy of species A; $\boldsymbol{n_pA}$ - the number of samples in which the species A is present;
$\boldsymbol{N_p}$ - total number of investigated samples.
Dominance (D) is considered to be an indicator of productivity, expressing the relationship of the population of a species to the sum of other associated species. Thus, this indicator shows the percentage of participation of the species in the catches. Dominance (D) was calculated using the relation:

$$\boldsymbol{D_A = \frac{n_A}{N} \times 100}, \text{ where:}$$

$\boldsymbol{D_A}$ - species dominance; $\boldsymbol{n_A}$- total number of individuals belonging to species; $\boldsymbol{A}$, present in the samples researched; $\boldsymbol{N}$ - total number of individuals belonging to all the species present in the samples researched.
The ecological significance index (W) represents the relationship between the structural indicator (C) and the productivity indicator (D), reflected by following formula used:

$$\boldsymbol{W_A = \frac{C_A \times D_A \times 100}{10000}}, \text{ in which:}$$

$\boldsymbol{W_A}$ - ecological significance index of the species A; $\boldsymbol{C_A}$ - species constancy; $\boldsymbol{D_A}$ - species dominance.

On each harvest date, the biological material captured from each trap was separated from the fixative liquid and placed in boxes with a 40% alcohol solution to preserve the elasticity of the insects and anesthetize the live ones. Further, each trap was reinserted in the soil and the fixative fluid be replaced. The collected entomological material was brought to the laboratory, and the insects were determined and Inventoried with the help of Reitter (1908) and Panin (1952) determinants.

Also, during this study the air temperature and rainfall was recorded by AgroExpert system, located in the experimental field.

RESULTS AND DISCUSSIONS

The observations and determinations were carried out in 2022 in the aronia plantation where the diversity and distribution of arthropods existing in this biotope was studied. Their dynamics, diversity and distribution are directly and indirectly affected by the variations of abiotic factors (Majed et al., 2020). Thus, during the study period, an average annual temperature of 11.4°C and an amount of precipitation of 379.0 mm were recorded, with a thermal deviation of +1.2°C and a precipitation deficit of -183.6 mm compared to the multiannual averages (Figure. 1).

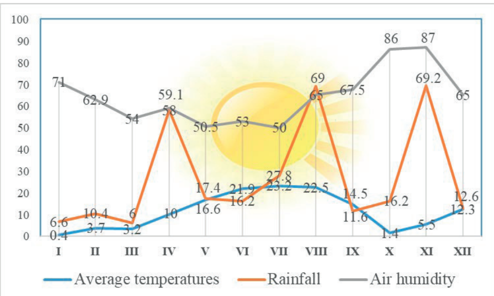

Figure 1. Dynamics of climate factors in 2022 at RSFG Iași

The collections of the arthropods using Barber traps were done on the following dates: 20.05.2022; 06.06.2022; 20.06.2022; 01.07.2022; 15.07.2022; 29.07.2022; 12.08.2022; 26.08.2022; 15.09.2022. During this period, a number of 3715 specimens of arthropods belonging to the *Insecta* and Arachnida classes were collected, distributed in the following orders: *Araneae*, *Coleoptera*, *Collembola*, *Dermaptera*, *Diptera*, *Hemiptera* with suborders *Heteroptera* and *Homoptera*, *Hymenoptera*, *Isopoda*, *Orthoptera*.

The *Coleoptera* order had the largest share with a total number of 1193 captured specimens belonging to 57 species. The order Hemiptera had 628 specimens of which 360 were of the suborder *Heteroptera* and 268 of the suborder *Homoptera*. The following representatives were the order Isopoda with 435 specimens; *Araneae* with 421 specimens, *Hymenoptera* with 365 specimens. The orders *Diptera* and *Orthoptera* recorded 223, respectively 175 specimens during the study. The presence in the form of a colony of species from the *Collembola* order made it impossible to calculate the number of specimens from the nine harvests. In the centralized table no. 1, the dynamics of catches during the study period can be observed, directly influenced by various factors: temperature, humidity, inter-specific relationships. Thus, the highest abundance of species was recorded at the third harvest, on 20.06.2022, with a number of 541 specimens. In this sample, the Coleoptera order had the largest share with a number of 248 specimens. From this order, the species *Harpalus distingunedus* Duft. and *Pterostichus cupreus* L. were the most abundant with 97 and 22 specimens, respectively. The next two samples recorded a number of specimens of 539 and 477, respectively, the taxa belonging to the orders *Coleoptera*, followed by *Araneae* and *Heteroptera*, to 208 and 34 species, respectively and taxa, respectively. The seventh harvesting date (12.08.2022) registered the lowest sample of arthropods in a number of 255 specimens, of which the greatest abundance was an isopod with 35 specimens of *Armadillidium vulgare* L., followed by the orders *Homoptera*, *Araneae*. Correlating the climatic factors (Figure. 1) with the dynamics of arthropods collected in the nine times (Table 1), it can be observed that there is a significant influence on the density and structure of the arthropod communities in the aronia crop. The density of arthropods varied over the growing season. Most arthropod groups were abundantly found in the spring-summer growing season. This is also confirmed by other research on arthropod distribution in different microenvironments along growing season on shrubs and various plants (Rango, 2005; Liu et al., 2013; Majeed et al., 2020). In the present study a significant positive relationship was found between arthropod diversity and temperature, with mouth average between 16.2°C and 23.2°C in June and July. Humidity showed a significant negative relationship with the diversity of entomofauna, on August was 69.0 mm of precipitation, with an excess of 18.2 mm compared to the multiannual average.

Table 1. The arthropods collected from the aronia plantation with the soil traps Barber method at RSFG Iași in 2022

No.	Order	Specie	No of harvesting									Total samples
			I	II	III	IV	V	VI	VII	VIII	IX	
1.	Araneae		58	45	55	57	56	27	35	52	36	421
2.	Coleoptera	*Amara aenea* De Geer			13	8	10	6				37
		Amara crenata Dejean	15	1								16
		Anisodactylus binotatus F.			3		6					9
		Anthicus floralis L.	2	2	3	6	7	2				22
		Anthicus humeralis Gebler				2	1		3			6
		Apion tenue K.	3									3
		Apion violaceum Gyll.			2	2						4
		Brachinus crepitans L.	1	2	5					10		18
		Brachinus explodens Duft.				2		3		1		6
		Brachinus psophia Serv.								1	1	2
		Cantharis violacea Thun.	2									2
		Carabus coriaceus L.			4				1	3	1	9
		Cartodere elongata Curt.	1									1
		Coccinella 12 punctata L.							1			1
		Coccinella septempunctata L.	2	2								4
		Corticaria longicornis Herbst	1									1
		Cymindis vaporariorum L.	2	1								3
		Dermestes laniarius Illi.	5	12	36	15	9		3	6		86
		Formicomus pedestris Rossi				3		1				4
		Harpalus distinguendus Duft.	65	78	97	62	10	2		37	35	386
		Harpalus aeneus F.				2	2			26	14	44
		Harpalus azuereus F				2				1	1	4
		Harpalus calceatus Duft.	1		12	15	3	4	11	25	17	88
		Harpalus griseus P.	10	18				1	1	23	14	67
		Harpalus picipennis Duft.	1									1
		Harpalus pubescens Müll	1		10	19	56	7	3	44	14	154
		Harpalus rufipes Deg.					18			9		27
		Harpalus tardus P.	2						2	4		8
		Hister funestus E.		1	4							5
		Hister neglectus G.	4		10							14
		Hister purpurascens Herbst.	2	5								7
		Leistus rufescens F.				1						1
		Longitarsus brunneus Duft.					1	1				2
		Longitarsus luridus S.								1		1
		Malachius bipustulatus L.		1	2							3
		Medon melanocephalus L.								1		1
		Ontophagus ovatus L.				2						2
		Opatrum sabulosum L.	20		19		23					62
		Otiorhynchus fuscipes Oliv.	3									3
		Otiorhynchus sensitivus S.			1							1
		Oxypora vittata L.						1				1
		Oxytelus rugosus F.								2		2
		Oxytelus sculpturalis Grav.								1		1
		Phyllotreta nigripes F.								1		1
		Phyllotreta vittula R.					2					2
		Podagrica malvae Illi.	1									1
		Pterostichus cupreus L.			22	9				4	5	40
		Pterostichus niger Sch.			1	3	4		2			10
		Pterostichus signatus L.				1						1
		Pteryngium crenatum Gyll.	1	1						1		3
		Scymnus frontalis F.						1				1
		Staphylinus erythropterus L				2						2
		Staphylinus pubescens De Geer.				1						1
		Tachyporus hypnorum F.	2		3							5
		Tachyporus nitidulus F	1		1							2
		Thiasophila inquilina Märkel	2									2
		Valgus hemipterus L.	1			2						3
3	Collembola		51	38	colony	43	59	colony	colony	29	34	254
4	Dermaptera				8	4			2	7		21
5	Diptera		23	25	47	32	21	16	20	15	24	223

No.	Order	Specie	No of harvesting									Total samples
			I	II	III	IV	V	VI	VII	VIII	IX	
6	Hemiptera	Heteroptera	69	36	67	76	48	5	12	40	7	360
7		Homoptera	26	18	20	31	57	45	39	20	12	268
8	Hymenoptera		47	38	31	62	41	46	35	40	25	365
9	Isopoda		25	34	47	39	52	68	77	56	37	435
10	Orthoptera		16	22	18	35	17	25	11	17	14	175
Total			440	380	541	539	477	261	255	470	291	3715

From another perspective, the ecological parameters of each experimental variant were analyzed in the study. Thus, variants 1 and 2 were treated with products allowed in ecological culture, and variants 3 and 4 with conventional products. The treatments for V1 and V2 including products based on copper, parafinic oil, natural plant extract such as Ovipron Top (0.2 %), Wetcit (0.25%), Laser 240 SC (0.06 %), Deffort (0.3 %), Funguran OH 300 SC (0.06%) to which biostimulants against biotic and abiotic stress were added (Mimox 0.2%, Altosan 0.25 %).
The conventional products for V3 and V4 treatments including Toil (0.5%), Mospilan (0.02%), Vertimec 1.8% EC (0.1%), Score 250 EC (0.03%), Merpan 80 WDG (0.15 %).

Figure 2. Aspects regarding the experimental plot and sampling

Within V1, the most abundant arthropods belong the *Coleoptera* order (371 specimens), following *Hymenopter*a group (217 specimens represinted by Formicidae and *Vespidae*, parasites entomophaguos) and *Isopoda* order (217 species). The mentioned orders are characterized in the categories of constant and euconstant species depending on the indicator (C) which expresses the continuity and appearance of the species in the analyzed biotope (Table 2). The accidental species (C1) encountered in this variant was represented by *Forficula auricularia* L. (*Dermaptera* ord.), which is considered also recedent species (D2), by dominance. According to the classification of ecological index W, the values were between 0.22% (*Dermaptera* ord.) and 36.37 (*Coleoptera* ord.).

Table 2. Ecological parameters analysis of the species collected in the aronia culture at V1

Order	(A)	(C)		(D)		(W)	
		%	Cl.	%	Cl.	%	Cl.
Araneae	102	85.18	C4	10.00	D4	8.52	W4
Coleoptera	371	100	C4	36.37	D5	36.37	W5
Dermaptera	12	18.51	C1	1.18	D2	0.22	W1
Diptera	14	25.92	C2	1.37	D2	0.36	W1
Heteroptera	76	55.55	C3	7.45	D4	4.14	W3
Homoptera	43	37.03	C2	4.22	D3	1.56	W3
Hymonoptera	217	70.37	C3	21.27	D5	14.97	W5
Isopoda	148	74.07	C3	14.51	D5	10.75	W5
Orthoptera	37	44.44	C2	3.63	D3	1.61	W3

Variant 2 (V2), in addition to the administration of organic treatments, it also benefited from irrigation, which changed the abundance and dominance of arthropod populations (Table 3). The abundance of *Coleoptera* increased to 402 specimens belong to the genre *Amara*, *Apion, Dermestes*, *Harpalus*, *Opatrum*, *Otiorhynchus*, following by *Isopoda* (217 specimens) and *Hymenomoptera* (196 specimens). In this sample the euconstant species (C4) belonged to orders *Araneae*, *Coleoptera* and *Isopoda* and the constant species (C3) are orders *Orthoptera* and *Hymenoptera*. The other orders are accessory species (C2) within the sample. Regarding the dominance, *Diptera* ord. was

included in the subreceding species (D1), *Dermaptera* ord. recordered in the receding species (D2). No group of arthropods is included in this variant as being subdominant, the rest that were not mentioned are either dominant (D4) or eudominant (D5). Ecological significance index recorded values between 0.43% (*Diptera*) and 34.44 (*Coleoptera*).

Table 3. Ecological parameters analysis of the species collected in the aronia culture at V2

Order	(A)	(C)		(D)		(W)	
		%	Cl.	%	Cl.	%	Cl.
Araneae	95	85.18	C4	8.11	D4	6.91	W4
Coleoptera	402	100	C4	34.33	D5	34.33	W5
Dermaptera	15	33.33	C2	1.28	D2	0.43	W2
Diptera	11	29.62	C2	0.94	D1	0.28	W2
Heteroptera	89	48.14	C2	7.60	D4	3.66	W3
Homoptera	78	40.74	C2	6.66	D4	2.71	W3
Hymonoptera	196	66.66	C3	16.74	D5	11.16	W5
Isopoda	217	92.59	C4	18.53	D5	17.16	W5
Orthoptera	68	74.07	C3	5.81	D4	4.30	W3

In the conventionally treated variants V3 and V4, the number of arthropods collected decreased significantly, under the action of the applied products. Thus, within V3, 759 specimens were analyzed. Compared to the previous versions, the number of coleopterans decreased, the sample having only 296 specimens. The index of constancy was between 14.81% and 92.59%. The most adapted species to the conditions of this biotope being *Coleoptera* (92.59 %), *Isopoda* (85.18 %) and *Hymenoptera* (81.48%) species. The dominance of arthropods varies between 0.92% (*Dermaptera*) and 39.00% (*Coleoptera*), while ecological significance index (W) has values between 0.14 % and 36.11 for the same orders (Table 4.).

Table 4. Ecological parameters analysis of the species collected in the aronia culture at V3

Order	(A)	(C)		(D)		(W)	
		%	Cl.	%	Cl.	%	Cl.
Araneae	47	48.44	C2	6.19	D4	3.00	W3
Coleoptera	296	92.59	C4	39.00	D5	36.11	W5
Dermaptera	7	14.81	C1	0.92	D1	0.14	W2
Diptera	19	44.44	C2	2.50	D3	1.11	W3
Heteroptera	75	70.37	C3	9.88	D4	6.95	W3
Homoptera	52	62.96	C3	8.17	D4	5.14	W4
Hymonoptera	95	81.48	C4	12.52	D5	10.20	W5
Isopoda	104	85.18	C4	13.70	D5	11.67	W5
Orthoptera	41	70.37	C3	7.11	D4	5.27	W4

Variant 4 (V4) was treated conventionally and irrigated. Thus, the results in table 5 show that compared to the previous version, the abundance of arthropods changed, with 788 specimens captured. It can be seen that humidity had a positive influence on the orders *Coleoptera*, *Diptera*, *Isopoda*, *Homoptera* and *Orthoptera.* The orders *Araneae, Dermaptera*, *Heteroptera* and *Hymenoptera* registered decreases regarding the number of specimens within this variant. This sample included all the classes from the point of view of the constancy. Regarding dominance values are between 0.76 % (*Dermaptera*) and 39.97 (*Coleoptera*) with the ecological significance index between 0.08-39.97%.

Table 5. Ecological parameters analysis of the species collected in the aronia culture at V4

Order	(A)	(C)		(D)		(W)	
		%	Cl.	%	Cl.	%	Cl.
Araneae	31	59.25	C3	3.93	D4	2.33	W3
Coleoptera	315	100	C4	39.97	D5	39.97	W5
Dermaptera	6	11.11	C1	0.76	D1	0.08	W1
Diptera	34	25.92	C2	4.31	D3	1.12	W3
Heteroptera	48	40.74	C2	6.09	D4	2.48	W3
Homoptera	61	48.14	C2	7.74	D4	3.73	W3
Hymonoptera	79	51.85	C3	10.03	D5	5.20	W4
Isopoda	173	92.59	C4	21.95	D5	20.33	W5
Orthoptera	54	70.37	C3	5.20	D4	3.66	W3

In Figure 3, the abundance of arthropods collected in the three experimental variants are graphically represented. It can be seen that the two experimental factors (applied treatments and irrigation) influenced the presence of epigean entomofauna. It is also observed that their number fluctuated between variants, but there were also variations within the collected orders.

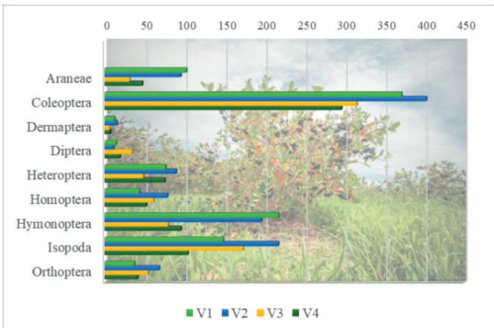

Figure 3. The abundance of arthropods collected in the experimental variants

CONCLUSIONS

In the study carried out, the structure and diversity of arthropods existing in an aronia plantation was investigated, within four experimental variants. In the period from May to September 2022 nine harvest of arthropods were recorded. thus, it was observed that the density of arthropods varied greatly over the growing season. Most arthropod groups were abundant found in the spring-summer growing season.

When performing the determination, inventory and structuring the specimens, it was observed that not only the abundance, but also the diversity of the species has a positive correlation with the structure of the vegetation. From another point of view, the seasonal change of the species found in chokeberry culture was influenced by abiotic factors, but also by inter-specific relationships.

The results obtained, but also the deepening of these studies, can be useful in the development of biological control strategies and the protection of aronia crops and others

Thus, it was concluded that the highest abundance was recorded within variants V1 and V2, respectively 1020 and 1171 belonging to the orders *Araneae, Coleoptera*, *Dermaptera*, *Diptera*, *Collembola, Heteroptera*, *Homoptera*, *Hymenoptera*, *Isopoda* and *Orthoptera.*

The application of conventional products influenced the density of arthropods in samples V3 and V4, their number being decreasing 736, respectively 788 comparing to the previous ones.

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THE ORIGIN OF ROMANIAN BLUEBERRY CULTIVARS

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Abstract

Vaccinium corymbosum (L.) is a plant native to North America and belongs to the genus Vaccinium L., the Ericales order, family Ericaceae, Subfamily Vaccinioideae. Blueberries are classified under the family Ericaceae, subfamily Vacciniaceae, genus Vaccinium, and subgenus Cyanococcus. They are most commonly found growing in acidic and infertile soil conditions. Highbush blueberries have a higher total sugar content compared to other berries of the Vaccinium genus. The first blueberry crop in Romania was established in Bilcesti, Arges. Since 1980, blueberry breeding has been carried out at the Research Institute for Fruit Growing Pitesti - Maracineni, Romania, using different origins and parent plants. Controlled hybridization is used in the breeding process, where the parents or genitors are chosen based on the desired blueberry breeding objectives. The aim of this study is to expand the knowledge base on the pedigree and biometrical traits of Romanian blueberry cultivars.

***Key words**: the first blueberry crop, controlled hybridization, pedigree, dendrogram of Vaccinium corymbosum.*

INTRODUCTION

Blueberries, belonging to the *Vaccinium* section *Cyanococcus*, are perennial shrubs that are extensively grown for their delicious fruits.
The *Vaccinium* genus comprises significant cultivated species like blueberry and cranberry (Kulkarni et al., 2020). Many *Vaccinium* species have been realized by blueberry breeders to develop cultivars adapted to diverse climates (Hanson et. al, 2007). Blueberries have a shallow root system, primarily located at depths less than 60 cm, especially on clay soils (Prodorutti et al., 2007). The species within this genus present different levels of ploidy (2*x*, 4*x* and 6*x*; *x* = 12), which results in evident morphological differences.
Vaccinium corymbosum (L.) (2 n=4×=48) is a tetraploid species and is the primary contributor to many highbush blueberry cultivars (Redpath L. et al., 2022). *Vaccinium* berries have gained tremendous attention in recent days due to the presence of a high amount of antioxidant properties (Sharma et al., 2022). Historical records indicate that the breeding of blueberries has deep roots in the incorporation of wild species and the crossing of different species (Brevis et al.,2008, Lyrene et al., 2012). The first blueberry breeder was Frederick V. Coville in Greenfield, America. He began observing the native highbush blueberries in the area in 1905 and started a blueberry breeding program using elite wild selections of *Vaccinium corymbosum* (L.). Coville, along with Elizabeth C. White, grew blueberries in plantations and released numerous crosses. Some of the breeding cultivars developed by F. V. Coville include 'Rubel' (wild selection in 1912), 'Pioneer' (1920), 'Katharine' (1920), 'Greenfield' (1926), 'Rancocas' (1926), 'Jersey' (1928), 'Concord' (1928), 'June' (1930), 'Stanley' (1930), 'Scammell' (1931), 'Redskin' (1932), 'Catawba' (1932), 'Wareham' (1936), 'Weymouth' (1936), and 'Dixi' (1936). After Coville died in 1937, researchers continued to release cultivars from his plantation and hybrids, including 'Angola' (1951), 'Atlantic' (1939), 'Berkeley' (1949), 'Bluecrop' (1952), 'Blueray' (1955), 'Burlington' (1939), 'Collins' (1959), 'Coville' (1949), 'Croatan' (1954), 'Earliblue' (1952), 'Ivanhoe' (1951), 'Murphy' (1950), 'Pemberton' (1939), and 'Wolcot' (1950). George Darrow continued his work through studies on the phylogeny of native species of *Vaccinium* (Darrow, 1952). In present, The National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, which is part of the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), is dedicated to preserving the

genetic resources of fruit crops. This genebank houses over 1800 accessions of Vaccinium L. from 34 different countries (Bassil et al., 2020). In Europe, Dr. Hermann from Germany made significant progress in blueberry breeding in 1929, establishing the first blueberry plantation in Germany and resulting in numerous cultivars. In Romania, blueberry breeding began in 1980 with Dr. Paulina Mladin at the Research Institute for Fruit Growing in Pitești - Mărăcineni. The breeding program continues to this day, focusing on meeting market requirements such as fruit size and extended ripening season. The program has resulted in a rich and diverse genetic material, with different stages of evaluation in the breeding process, including hybrids and comparative crops and microcultures.
That will serve to obtain new, competitive cultivars, as a new source for the continuation of the breeding program. The main objectives of breeders for highbush blueberry have been the selection of aromatic cultivars with the ability to be resistant to storage, fruiting period, disease and pest resistance and mechanized harvesting (Qu et al., 1998). The objectives of blueberry breeding are similar to those being realized in other breeding centres working with this crop (Pluta et al., 2012). As a working methodology, in particular, directed, controlled hybridization is used, using the rich collection from Pitesti and composed of 9 cultivars registered in the EURISCO European catalog: 'Azur' (1998), 'Safir' (1998), 'Augusta' (1999), 'Simultan' (2001), 'Delicia' (2001), 'Lax' (2002), 'Vital' (2009), 'Prod' (2009) 'Pastel' (2019).
The aim of this study is an expanded knowledge base of pedigree and biometrical traits for Romanian cultivars of blueberry.
Plant breeding success in the last century has been linked to a reduction in the genetic diversity present in the superior germplasm of plant species (Zoratti et al., 2015).

MATERIALS AND METODS

Plant material

The biological material was represented by 9 Romanian cultivars of blueberry (Figure 1): 'Azur' and 'Augusta cv.' originated from the cross 'Berkeley' × 'Bluecrop' cv.; 'Safir' cv. originated from the cross 'Pemberton' × 'Blueray' cv.; 'Simultan', 'Lax', 'Pastel' and 'Vital' cv. obtained from open pollination 'Spartan' cv.; 'Delicia' and 'Prod' obtained from open pollination 'Patriot' cv.

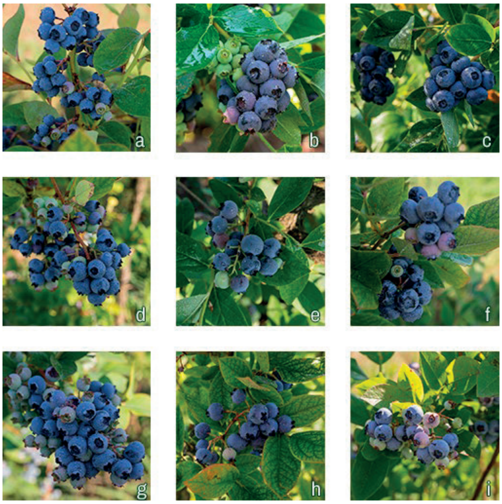

Figure 1. The biological material: a- 'Lax'; b-'Pastel'; c-'Safir'; d-'Vital'; e-'Prod'; f-'Delicia'; g-'Azur'; h-'Simultan'; i-'Augusta'

The experiment was set up at the Research Institute Growing Pitesti, within the Genetic and Breeding Department and tested in an experimental plot. The cultivars were planted at a distance of 3 m × 1 m on a mixture of soil and peat. The samples were harvested and immediately analyzed.

Determination of yield

By weighing total yield/plant (kg/plant).

Determination of Average Weight

By weighing a sample of 50 fruits for each genotype (15 plants/genotype), the average weight of fruits was determined and the results were expressed in g/fruit.

Determination of shape index

The length and diameter of the fruit were determined by measuring the fruit using a digital caliper. The shape index (SI) of the fruit was calculated as the ratio of these two dimensions (Tudor et al. 2014, Jamieson, 2017).

Determination of fruit firmness

By measuring each fruit sample with a penetrometer Bareiss HPE II Fff nondestructive test, with a measuring surface of 0.25 cm².

Determination of total titratable acidity

TTA was determined by titrable method (Ermakov et al., 1987). The principle of the method is to neutralize a volume of aqueous fruit extract with a solution of NaOH 0.1N in the presence of phenolphthalein as an indicator.

Determination of Total Soluble Solid Content

Total soluble solids (TSS) were determined using a Kruss DR201-95 refractometer and the results were reported as °Brix at 20°C.

Statistical Analysis

All analyses were performed in triplicate and data were reported as mean standard deviation (SD). Excel 2021 (XLSTAT) was used for data statistical analysis. One-way analysis of variance (ANOVA) and two-way ANOVA and Duncan’s multiple range tests were performed. The genetic distance between varieties was determined using Minitab v. 18 software based on the Euclidean distance formula, which calculates the square root of the sum of squared differences: dik=$\sqrt{\sum_j (Xij - Xkj)}\,^2$.

RESULTS AND DISCUSSIONS

The analysis of the dendogram indicates four groups: group I formed by the cultivars: 'Lax' and 'Augusta'; group II formed by the cultivars: 'Pastel', 'Safir' and 'Simultan', group III formed by the cultivars: 'Azur' and group IV formed by the cultivars: 'Vital', 'Prod' and 'Delicia' (Figure 6).

The smallest genetic distance is between the cultivars 'Safir' and 'Simultan', and the largest genetic distance is between the cultivars 'Lax' and 'Vital'. The greatest degree of similarity is between the cultivars 'Safir' and 'Simultan', 'Prod' and 'Delicia', 'Lax' and 'Augusta', 'Azur' cv. clearly differs from all other cultivars.

The biggest yield of the cultivars tested was registered by 'Azur' cv. (3.93 kg/plant) and 'Delicia' cv. (3.58 kg/plant).

The lowest yield (shown in Table 1) was recorded by 'Vital' cv. (1.77 kg/plant).

For average fruit weight, there were no significant differences between cultivars, the highest fruit weight was recorded by 'Delicia' cv (3.1 g).

Regarding fruit firmness, statistically significant differences were found between cultivars, and it is a trait that directly affects the quality of blueberries. The average fruit firmness ranged from 12.63 N (for the 'Azur' cultivar) to 38.4 N (for the 'Simultan' cultivar).

According to previous studies, the content of organic acids in fruits can be influenced by culture and environmental conditions (Gündoğdu, 2019), genotypic differences and post-harvest handling procedures (Lee and Kader, 2000). In this study, the richest content of organic acids expressed as citric acid was 1.22% (for the 'Azur' cultivar), and the lowest content was 0.72% (for the 'Augusta' cultivar).

Along with other phytochemical compounds, the soluble solids content contributes to fruit flavor. TSS consisting of soluble carbohydrates and other non-carbohydrate compounds influences the refractive index of the aqueous extract obtained from a horticultural product (Diaconu, 2006).

In our study, for total soluble solids content ranged from 8.93 °Brix for 'Lax' cv. to 19.3 °Brix for 'Simultan' cv. (Table 1).

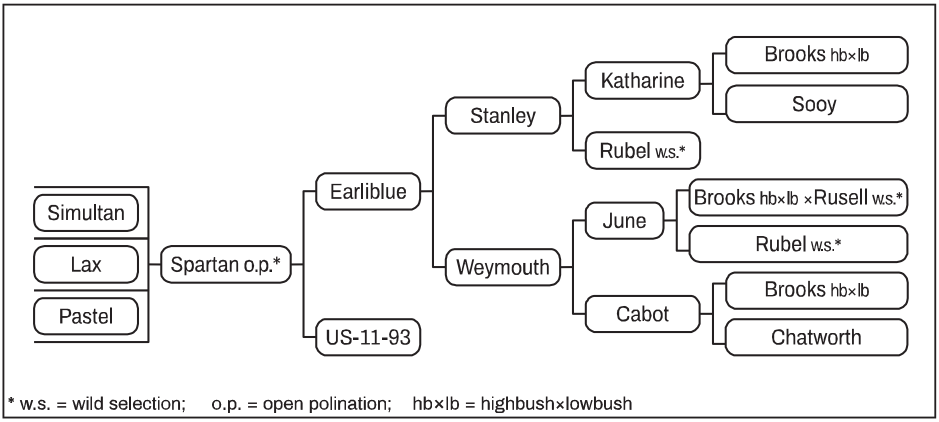

Figure 2. Pedigree of 'Simultan', 'Lax' and 'Pastel' cv. highbush blueberry

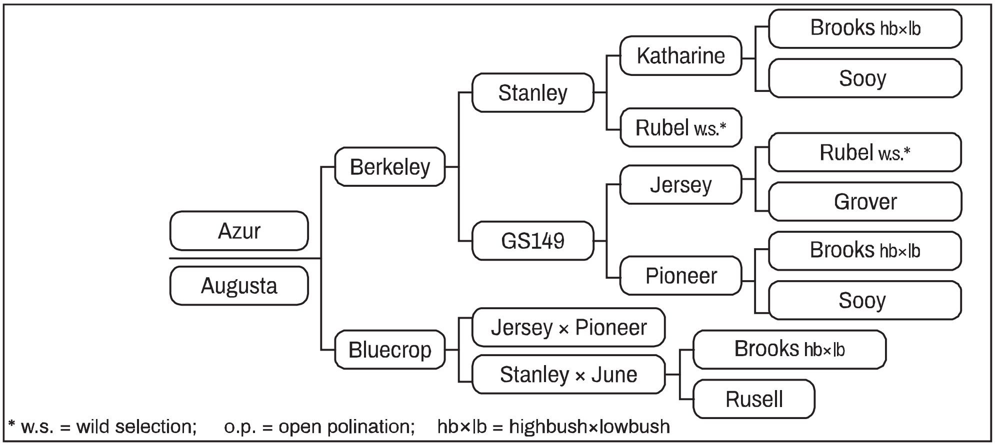

Figure 3. Pedigree of 'Azur' and 'Augusta' cv. highbush blueberry

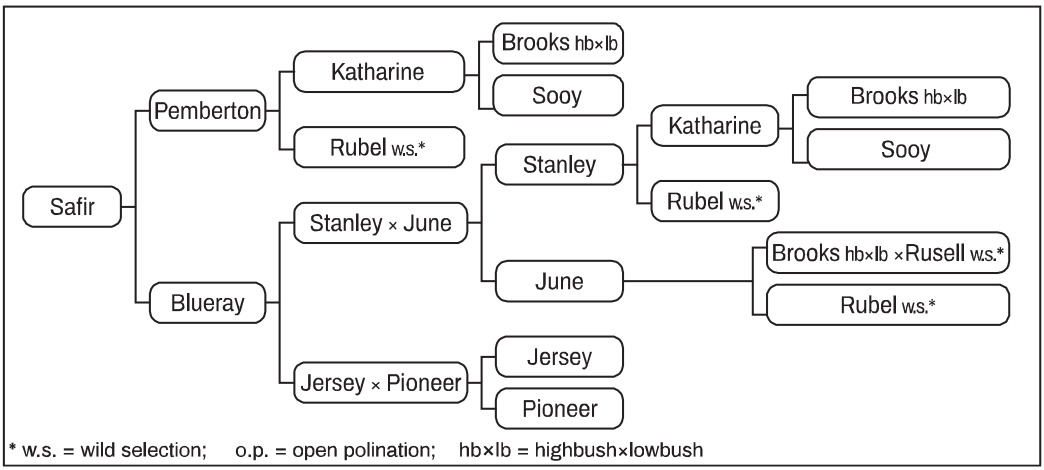

Figure 4. Pedigree of 'Safir' cv. highbush blueberry

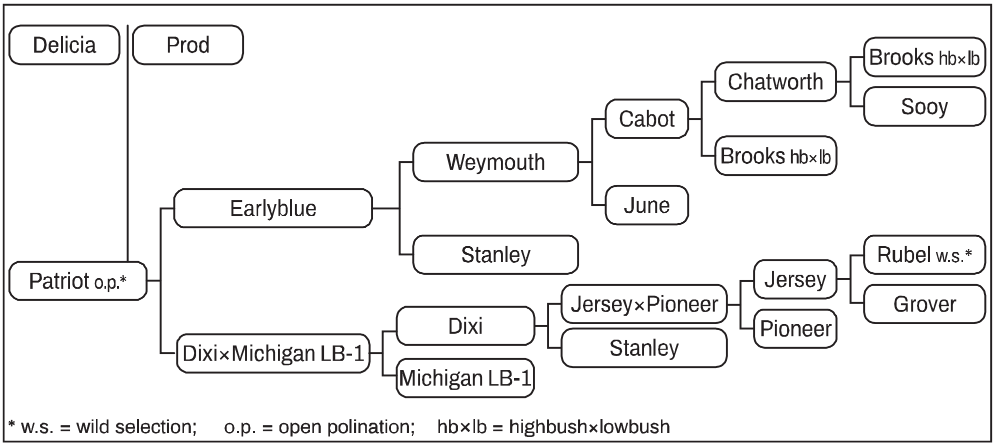

Figure 5. Pedigree of 'Delicia' and 'Prod' cv. highbush blueberry

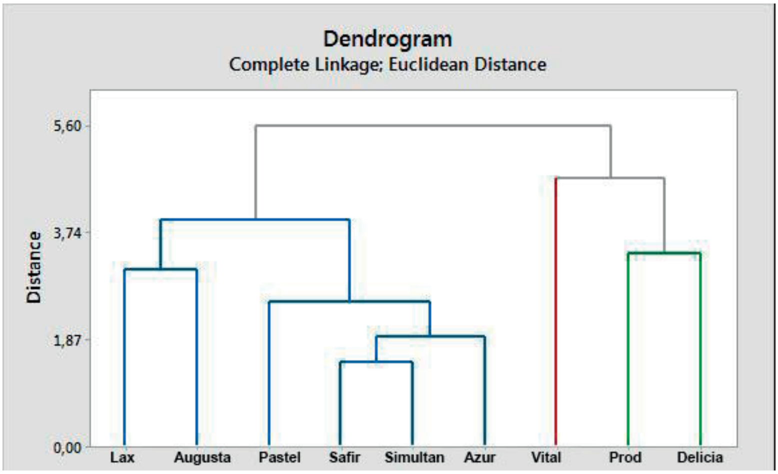

Figure 6. Dendrogram of 9 blueberry cultivars generated by Minitab v.18, cluster analysis of the Euclidean Distance based on biometrical traits

Table 1. The average values of yield, berry weight, size index, firmness, acidity and °Brix at 9 blueberry cultivars

Cutivar	Yield*	Berry Weight (g)	Size index	Firmess (N)	TTA (%)	TSS (° Brix)
Lax	3±0,2cd	2,57±0,76a	0,87±0,06a	20,73±7,1cd	0,75±0,13a	8,93±1,03b
Pastel	2,78±0,3c	2,99±0,71a	0,76±0,09bc	22,9±3,06bcd	1,25±0,5a	10,73±1,1b
Safir	2,67±0,48c	2,7±0,2a	0,71±0,09c	24±0,95bcd	0,91±0,15a	11,17±1,24b
Vital	1,77±0,25d	2,77±0,81a	0,72±0,02c	35,74±6,68ab	1,22±0,48a	9,58±0,65b
Prod	2,55±0,54c	2,47±0,51a	0,72±0,01c	28,93±10,89abc	0,7±0,08a	13,37±3,1b
Delicia	3,58±0,56ab	3,1±0,56	0,7±0,04c	28,63±3,7abc	0,93±0,07a	11,33±0,95b
Azur	3,93±0,12a	3,08±0,51a	0,88±0,04a	12,63±0,15d	1,04±0,67a	10,43±0,65b
Simultan	2,63±0,55c	2,4±0,52a	0,85±0,06ab	38,4±0a	1,19±0,4a	19,3±8,75a
Augusta	2,63±0,55c	2,03±0,32a	0,75±0,07bc	28,73±13,97abc	0,72±0,13a	10,87±1,5b

*Means with the same letter are not significantly different at 5% level

CONCLUSIONS

Dr. Coville's significant contribution to understanding the cultural requirements of blueberries is immeasurable. However, we can assess the value of the blueberries from the bushes he breeded. The cultivars created by Dr. Coville contributed to the breeding of the 9 Romanian blueberry cultivars. The Rubel cv., common ancestor, is a selection from the spontaneous flora in 1912.

The different cultivars he introduced and the seedlings he cultivated, which were later released, are illustrated in Figure 2 to Figure 5. This paper shows the broad origin of the nine Romanian blueberry cultivars.

According to the results presented in our study, the smallest genetic distance is between the cultivars 'Safir' and 'Simultan', and the largest genetic distance is between the cultivars 'Lax' and 'Vital'. The greatest degree of similarity is between the cultivars 'Safir' and 'Simultan', 'Prod' and 'Delicia', 'Lax' and 'Augusta', 'Azur' cv. clearly differs from all other cultivars.

The biggest yield of the cultivars tested was registered by 'Azur' cv. (3.93 kg/plant) and 'Delicia' cv. (3.58 kg/plant).

It is proposed to create a new *Vaccinium corymbosum* (L.) from these cultivars. Our results will strengthen the breeding program.

ACKNOWLEDGEMENTS

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COMERCIAL AND BIOCHEMICAL QUALITY OF ROMANIAN PEAR GENOTYPES

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Abstract

The present study was carried out to evaluate the quality of different pear genotypes used in the breeding programs at Genetic and Breeding Department of Research Institute for Fruit Growing Pitești, Romania. Thirteen new pear genotypes harvested at the commercial maturity stage: 'Argessis', 'Carpica', 'Cristal', 'Daciana', 'Haydeea', 'Isadora', 'Paradox', 'Paramis', 'Romcor', 'P20R41P30', 'SP06C2P', 'Triumph' and 'Tudor' were compared with internationally recognized varieties: 'Monica' (Romanian cultivar), 'Xenia' and 'Williams'. In this study we analyzed the commercial parameters (weight, firmness, color) and the biochemical indicators (total dry mater content, soluble dry matter, titratable acidity, the soluble dry matter/ titratable acidity ratio, vitamin C, and polyphenols). The obtained data leads to the conclusion that 'Isadora' and 'SP06C2P5P' fruits have the biggest chance to fulfill consumer expectations, due to their commercial qualities, then the reference variety 'Williams' cultivar. Also, the 'Isadora' variety had the content of polyphenols and the total dry substance superior to the three control cultivars.

***Key words**: breeding program, fruit quality, polyphenols, Pyrus communis (L).*

INTRODUCTION

Pyrus communis L. is a typical crop of temperate climates and its fruit has numerous health benefits (Piluzza et al., 2023). It is an important and valuable fruit tree species, due to the agrobiological characteristics of the trees, the taste quality of the fruits, their nutritional and therapeutic properties, representing one of the main fruit crops of temperate climate (Sestraş, 2004).

Pear is cultivated on all continents, both in the Northern and Southern hemispheres, at the level of 2022, totaling a production over 26 million tons (FAOSTAT 2024). It gives the best results in regions with a temperate climate, but also succeeds well in subtropical regions.

Fruit quality is the main objective of all fruit breeding programs and encompasses a wide range of characteristics. A good quality fruit for the consumer an appropriate texture is needed, with balanced sweet and sour taste, and full development of typical pear flavor. In general, pears with external appearance of a whole fruit are used as an indicator of ripeness, although it can be a misleading one (Shewfelt, 2000). For consumers, the external appearance is very important, which refers to the size, shape, firmness and color of the fruit. Besides the commercial aspect, a major role is played by the taste and the other intrinsic characteristics of the fruit (aroma, juiciness, consistency, lack of sclereids). This set of quality elements is given, on the one hand, by the genetic structure of each variety, and on the other hand by the environmental conditions and the culture technology applied.

Pear (*Pyrus communis* L.) fruit is rich in health promoting antioxidant compounds such as phenolic compounds (Ceccarelli et al., 2021; Wang et al., 2021). The composition of organic acids in pears is more variable than that of sugars. Generally, the most abundant is malic acid in all cultivars, but Drake and Eisele (1999) found very high proportion of citric acid in 'Bartlett' pears. Malic and shikimic acid decreased during ripening. Ripening is a key factor for fruit quality.

Branişte and Rădulescu (1994) showed as possible sources of genes for improving the quality of pears, the varieties 'Highland', 'Beierschmidt', 'Delbarexquise d'hiver', 'Graslin', 'Napoca', 'Untoasa de Geoagiu', 'Argessis' (for good taste), 'Triomphe de Jodoigne', 'President Heron', 'Beurre d'Anjou', 'Bergamotte Esperen', 'Notair Lepin', 'Grand Champion' (for high sugar content), 'Alexandre Lucas', 'Thompson', 'President Drouard', 'Matya', 'Moonglow', (for high vitamin C content).
The objective of this study was to evaluate different pears cultivars used in the breeding programs at Genetic and Breeding Department of Research Institute for Fruit Growing Pitești - Maracineni, Romania.

MATERIALS AND METHODS

Field trial and plant material

The study was carried out at Research Institute for Fruit Growing Pitesti - Maracineni, Genetic and Breeding Department, during harvest season 2023. Change in fruit quality parameters at different origin pears cultivars: 'Argessis', 'Carpica', 'Cristal', 'Daciana', 'Haydeea', 'Isadora', 'Paradox', 'Paramis', 'Romcor', 'P20R41P30', 'SP06C2P5', 'Triumf', 'Tudor, 'Monica' from Romania, 'Xenia' from Republic of Moldova and 'Williams' from UK, were evaluated by point of view commercial and biochemical quality of fruits. As control, the commercial variety 'Monica' (Ct1), 'Xenia' (Ct2) and 'Wiliams' (Ct3) were used. The trees grafted on M9, were planted in spring 2009, at 3.5 meters between rows and 2 m between trees. The optimal harvest time for picking each cultivar was constructed on the basis of reports in the pomological literature and for internal quality of fruits (Table 1).

Soil Description

The most favorable conditions for pear are found on soils with medium texture (clay-sandy and clay-argued), which provides optimal retention, transfer and movement regime in the soil, of retention and transfer of nutrients, optimal cationic exchange capacity.
The experimental plots are located on the third terrace of the Arges river, the type of soil being clear cambic (formerly brown eumezobasic), with sandy-clay texture up to 80 cm in-depth. The main agrochemical indicators that characterize the soil are the following: the pH in the water is included 6.16-6.36 (moderately acid), slightly under the requirements of the species compared to this aspect; The humus reserve has values between 0.86 and 2.11%; the nitrogen index has very low values (0.04-0.10%) the soil falling into the category of those with poor nitrogen supply.

Climatic conditions

The studied area has a humid temperature continental climate, climatic conditions of Maracineni, Arges are characterized by the average annual temperature of 12.3°C, the average annual rainfall of 663.3 mm and the relative humidity of the air wanted the value of 79.6%.
For the pear, the vegetation period is longer, between 180 and 270 days, compared to only 170 - 210 at the apple (Coman et al, 2014).

Measurements

To assess external fruit quality at harvest, ten representative pear fruits of each cultivar were used for physical and chemical analysis by standard methods:
- Fruit weight was determined by weighing a sample of ten fruits (g/fruit);
- The firmness of the fruit was determined with a HPE non-destructive penetrometer with a 0.5 cm^2 measuring device, expressed in HPE units (from 0 - without firmness - to 100 - very hard), the measurement was performed on the two parts of the fruits according to ECPGR, Pear (*Pyrus communis*) recommendations, 2022;
- The fruit content of the acids (malic, citric and tartaric acid), expressed in g / 100 g of fresh pulp or in percent, was determined by the Mini Titrator -Hanna Instrument 84532;
- The color of fresh fruits is one of the most important sensory quality attributes. The color of food surface is the first quality parameter evaluated by consumers, and it is critical to product acceptance. Fruits appearance determined mostly by surface color is the first sensation that the consumer perceives and uses as a tool to either accept or reject food (Leon et al., 2006).

Table 1. Background information of pear cultivars studied

Cultivar	Reported parentage	Institution	Tree	Fruit	Production	Harvesting time
Argessis	Napoca x Butira precoce Morettini	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Medium size (125 g), pyriform, regular shape	Yields are constant	August
Carpica	Napoca x Butira precoce Morettini	Research Institute for Fruit Growing Pitesti, Romania	Medium - strong vigor	Medium-sized, on average 140 g, pyriform	High productivity	August
Cristal	[(Rosior pietros x Doyenné du Comice)xDoyenné du Comice]x Beurré Hardy	Research Station for Fruit Growing Voinesti, Romania	Medium vigor	Medium-large size, on 200 g average, the conical shape	High yielding potential	October-November
Daciana	Napoca x Butira precoce Morettini	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Medium sized (150g), pyriform shape	High productivity	July
Haydeea	Beurré Hardy x Beurré Six	Research Station for Fruit Growing Cluj, Romania	Medium growth vigour	Medium-large size, on 180 - 220 g	High productivity (over 40-50 t/ha)	September
Isadora	Haydeea x Tse Li	Research Institute for Fruit Growing Pitesti, Romania	Medium –strong vigor	Medium sized, 115g	High productivity	October
Paradox	Monica x Pastravioare	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Medium to large size, 150-170 g	Yields are constant	October
Paramis	Monica x Passe Crassane	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Large, on average 180 g	Good productivity	September
Romcor	[Passe Crassane x (*Pyrus serotina* x Olivier de Seres)] x Doyenné du Comice	Research Station for Fruit Growing Voinesti, Romania	Medium - strong	Medium to large size, 200 g on average	Good productivity	October
SP06C2P5	Packham's Triumph x Monica	Research Institute for Fruit Growing Pitesti, Romania	Medium –strong vigor	Medium to large size	High yielding potential	September
P20R41P30	unknown	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Medium to large size	Good productivity	October
Triumf	Napoca x Beurré Giffard	Research Institute for Fruit Growing Pitesti, Romania	Small to medium vigor	Medium (120g), pyriform shape	Good productivity	July
Tudor	[(*Pyrus serotina* x Doyenné du Comice) x Passe Crassane] x 30-44 Angers	Research Institute for Fruit Growing Pitesti, Romania	Medium - strong vigor	Large (200-250 g)	High yield potential	September-October
Monica (Ct_1)	Santa Maria x Principe di Gonzaga	Research Institute for Fruit Growing Pitesti, Romania	Medium vigor	Medium to large size, between 160-180 g	High productivity	September-October
Xenia (Ct_2)	Triomphe Vienne x Doynné Krier	Scientific-Practical Institute of Horticulture and Food Technologies Chisinau, MD	Medium vigor	Large (300-250 g)	High yield potential	October
Williams (Ct_3)	unknown	Aldermaston, UK	Medium vigor	Medium to large size (150-250 g)	Good productivity	September

The fruit skin color was visually evaluated and also with the Konica Minolta CR 400 colorimeter in the system (La*b*). The CIE (Commision Internationale de L'Eclairage)

LAB color range is an approximately uniform color scale in which the color space is organized in the shape of a cube. The L* axis is executed from top to bottom, representing a measure of brightness, on a scale from 100 (completely transparent) to zero (completely opaque). Axes a* and b* do not have specific numerical limits. Positive values to a* show red color and negative values show green color. The positive values for b* show the yellow color, and the negative values for the blue color (Chivu et. al, 2018).

pH-indicates the acidity or alkalinity of a substance, is a crucial metric for assessing the quality and ripeness of fruits, including pears(Taghinezhad et.al, 2023).These vary depending on the varieties, the pedoclimatic conditions and agrotechnics apply in orchards.

Total soluble solids content (TSS) was measured with Atago Palette PR32 digital refractometer (0-32°Brix).

The fruit content of the acids (malic, citric and tartaric acid), expressed in g / 100 g of fresh pulp or in percent, was determined by the Mini Titrator -Hanna Instrument 84532.

Total content in dry weight (DW) % was determined by the gravimetric method, by keeping the fruit tissue at 105°C until constant weight (AOAC, 2002).

Total polyphenolic content (mg GAE/100g FW) was assessed spectrophotometrically by measuring the optical density of the alcoholic fruit extract complexes with the Folin-Ciocalteu reagent (Singleton et al., 1999). The determinations were compared with a standard solution of gallic acid and the results were expressed in mg GAE/kg fresh fruit (Escribano – Bailon & Santos – Buelga, 2003).

Vitamin C content (mg/100 g FW) was determined according to the method based on the oxidation of L-ascorbic acid to dehydro-ascorbic acid in acidic medium (PN-A-04019, 1998). The vitamin C content (mg/100 g FW) is considered to be a very important quality indicator, because ascorbic acid is a bioactive compound with antioxidant properties.

Statistical analysis

The experiment was conducted in each sample and the data are expressed as mean ± standard deviation (SD). All data was normally distributed and respected homogeneity of variance. Results were processed by Excel (Microsoft Office 2010) and SPSS Trial Version 14.0. Data were subjected to analysis of variance (One-way ANOVA; $p \leq 0.05$), and Duncan’s Multiple Range Test (DMRT) post hoc tests were used to measure specific differences between sample means.

RESULTS AND DISCUSSIONS

From the study of the results, it turned out that the limits of the variability of the main biochemical components that define the quality are very wide, from double to triple and even more.

Thus, in the varieties with the maturation time in the summer months, the analyzed components oscillate within: 89.33-159.33 g weight; 51.9-65,21 mm caliber; 49.03-84.97 HPE units firmness; 11.53-16.37% SSC; 3.31-3.65 pH; 0.18-0.33% malic acid; 0.17-0.28% citric acid; 0.20-0.36% tartaric acid; 1879.71-3114.49 mg GAE/kg FW polyphenols; 83.51-85.79% water content; 14.21-16.49% DW; 3.49-9.25mg/100g FW vitamin C; 42.04-63.43 L; -16.17-7.43 a*; 15.3-29.53 b*.

At the varieties with harvesting in the autumn months the analyzed components oscillatedwithinthe limits: 152.67-385.33 g weight; 63.14-91.45 mm caliber; 49.03-81.58 HPE units firmness; 12.53-15.83% SSC; 3.32-4.37 pH; 0.19-0.50% malic acid; 0.16-0.50% citric acid; 0.19-0.59% tartaric acid; 1010.15-4027.53 mgGAE/kg FW polyphenols; 79.49-85.35% water content; 14.24-20.51% DW; 4.46-10.3125 mg/100 g FW vitamin C; 45.59-66.9 L; -12.41-14.62 a*; 19.12-33.62 b*.

Fruit weight

Regarding the fruit weight, that could be taken as an indicator of potential consumer appreciation of the fruit at the market. From a genetic point of view, the weight of the pears is a polygenic character, whose manifestation is strongly influenced by the environmental conditions (Sestraș, 2004). Thus, following the statistical analysis of the data, it was observed that the varieties studied were in homogeneous classes, the average fruit weight ranging between 89.33 g in the 'Triumf' variety to 385.33 g in the 'Paramis' variety. The varieties 'Paramis' and 'Tudor' overcoming control cultivars taken in study: 'Monica' (214 g), 'Williams' (253.33 g) and 'Xenia' (206.00 g) (Table 2).

Caliber

The limits between which the fruits of the new varieties must be located, to that optimal size, are between 150-250 g (but as uniform, without variability inside the variety), which corresponds to a diameter of 6-7.5 cm (Sestraș, 2004). The studied varieties were within this limit, except 'Paramis' cv. (92.92 mm), 'Tudor' cv. (91.45 mm) and 'Triumf' cv. (51.9 mm) (Table 2).

Table 2. Fruit characteristic of the pear genotypes

Genotype	Weight (g)*	Caliber (mm)*	Firmness (HPE units)*
Argessis	126.67 ± 20.43^{ef}	60.5 ± 4.30^{e}	84.97 ± 1.06^{a}
Carpica	159.33 ± 6.43^{cdef}	65.21 ± 2.52^{cde}	83.17 ± 2.49^{a}
Cristal	152.67 ± 11.02^{def}	66.18 ± 2.04^{cde}	71.6 ± 1.39^{cdef}
Daciana	153.33 ± 9.02^{def}	$61.42\pm3.6d^{e}$	83.07 ± 1.55^{a}
Haydeea	193.33 ± 4.16^{bcde}	67.26 ± 1.26^{cde}	75.2 ± 6.60^{abcde}
Isadora	228.67 ± 108.71^{bc}	69.62 ± 9.87^{de}	77.32 ± 4.32^{bcdef}
Monica (Ct_1)	214.00 ± 22.54^{bcd}	66.9 ± 3.32^{bcd}	50.8 ± 0.92^{abcd}
P20R41P30	172.00 ± 20.3^{cde}	63.14 ± 2.12^{bc}	72.62 ± 12.55^{g}
Paradox	176.67 ± 7.02^{cde}	67.76 ± 1.92^{cde}	65.3 ± 7.34^{f}
Paramis	385.33 ± 68.71^{a}	92.92 ± 9.67^{bcde}	76.1 ± 1.49^{abcde}
Romcor	201.33 ± 14.47^{bcd}	73.12 ± 3.89^{a}	66.93 ± 2.80^{ef}
SP06C2P5	223.67 ± 24.01^{bcd}	74.08 ± 1.96^{bc}	80.4 ± 3.61^{abc}
Triumf	89.33 ± 2.52^{f}	51.9 ± 6.50^{f}	49.03 ± 0.94^{g}
Tudor	375.33 ± 64.79^{a}	91.45 ± 3.93^{a}	69.98 ± 0.97^{def}
Williams (Ct_3)	253.33 ± 21.20^{bc}	76.47 ± 3.85^{a}	25.8 ± 10.71^{h}
Xenia (Ct_2)	206.00 ± 7.21^{bcd}	70.42 ± 1.74^{bcd}	81.58 ± 1.55^{ab}

*Values followed by the same letter are not statistically different according to DMRT (P<0.05).

Firmness measurements

Fruit firmness is an important criterion for fruit quality pear (De-Ell et al., 2001) and the loss of fruit firmness is a serious problem in postharvest handling pear fruits (Kov & Felf, 2003), because it resulted in soft and mealy fruit with poor consumers acceptance. Firmness is a main quality indicator of the pear flesh and is commercially used to predict the optimal harvest date of pear (Hic et al., 2023; Wang, 2015). Torregrosa in 2019 showed that consumers most appreciated pears of the 'Conference' variety, which showed firmness value in the range of 10-30 N. The maximum average of flesh firmness (84.97 HPE units) was registered at 'Argessis' cv., follow by 'Carpica' with 83.17 HPE units. The smallest value of the firm of firmness was at the 'Williams' (25.8 HPE units) variety used as at control. 'Isadora' cv., traits inherited from the species *Pyrus serotina*, one of the parents of this cultivar belonging to this species (Militaru et al., 2010) also had high firmness (Table 2).

Total soluble solids content (TSS)

Sugar, but also, acids and volatile substances are involved in taste and flavor of pears (Maresi et al, 2022). The summer cultivars generally had much lower soluble solids content than the late season ones, what confirms our study. The soluble solids content of fruits varied between 11.16% Brix and 16.37% Brix.

Table 3. The chemical properties of the fruit in the pear genotypes studied

Genotype	TSS (%Brix)*	pH*	Titratable acidity (%)		
			Malic acid*	Citric acid*	Tartaric acid*
Argessis	$13.57\pm$ 0.85de	3.62 ± 0.02^{ef}	0.20 ± 0.02^{jk}	0.19 ± 0.02^{ij}	0.23 ± 0.02^{i}
Carpica	13.2 ± 0.36^{def}	3.54 ± 0.09^{efg}	0.33 ± 0.03^{f}	0.30 ± 0.02^{f}	0.36 ± 0.03^{f}
Cristal	13.87 ± 0.32^{cde}	3.78 ± 0.12^{de}	0.28 ± 0.00^{h}	0.27 ± 0.00^{g}	0.31 ± 0.00^{g}
Daciana	11.53 ± 0.25^{f}	3.31 ± 0.12^{fg}	$0.29\pm0{,}02^{h}$	0.28 ± 0.02^{g}	0.32 ± 0.02^{g}
Haydeea	15.83 ± 2.83^{ab}	4.13 ± 0.29^{abc}	0.21 ± 0.00^{j}	0.20 ± 0.00^{i}	0.23 ± 0.00^{i}
Isadora	15.53 ± 0.90^{abc}	4.43 ± 0.11^{a}	0.17 ± 0.00^{i}	0.16 ± 0.00^{m}	0.19 ± 0.00^{k}
Monica (Ct_1)	15.5 ± 0.30^{abc}	3.63 ± 0.20^{ef}	0.30 ± 0.01^{m}	0.29 ± 0.01^{f}	0.34 ± 0.01^{f}
P20R41P30	12.63 ± 0.60^{def}	3.75 ± 0.19^{de}	0.25 ± 0.00^{g}	0.24 ± 0.00^{h}	0.28 ± 0.00^{h}
Paradox	12.9 ± 1.31^{def}	4.03 ± 0.11^{cd}	0.35 ± 0.00^{e}	0.33 ± 0.00^{e}	0.39 ± 0.00^{e}
Paramis	12.53 ± 0.15^{ef}	3.60 ± 0.38^{ef}	0.40 ± 0.00^{d}	0.39 ± 0.00^{d}	0.45 ± 0.00^{d}
Romcor	14.5 ± 0.36^{bcd}	3.25 ± 0.2^{1g}	0.5 ± 0.01^{b}	0.47 ± 0.01^{b}	0.55 ± 0.01^{b}
SP06C2P5	13.23 ± 1.20^{def}	4.06 ± 0.16^{bcd}	0.53 ± 0.00^{a}	0.5 ± 0.00^{a}	0.59 ± 0.00^{a}
Triumf	16.37 ± 0.15^{a}	3.65 ± 0.24^{ef}	0.18 ± 0.02^{lm}	0.17 ± 0.02^{km}	0.2 ± 0.02^{jk}
Tudor	13.1 ± 1.14^{def}	4.21 ± 0.16^{abc}	0.19 ± 0.00^{kl}	0.18 ± 0.00^{jk}	0.21 ± 0.00^{j}
Williams (Ct_3)	13.73 ± 0.61^{cde}	3.32 ± 0.15^{fg}	0.45 ± 0.0^{c}	0.43 ± 0.01^{c}	0.5 ± 0.00^{c}
Xenia (Ct_2)	13.9 ± 0.2^{cde}	4.37 ± 0.15^{ab}	0.17 ± 0.00^{m}	0.16 ± 0.00^{m}	0.19 ± 0.00^{k}

*Values followed by the same letter are not statistically different according to DMRT (P<0.05).

The highest average of TSS was registered at 'Triumf' cv. with 6.37% Brix, followed by 'Haydeea' cv. with 15.83% Brix. Making a comparison between the values of the soluble solid content, it should be mentioned that the following genotypes studied had a lower content in the soluble solid than the control cultivars: 'P20R41P30', 'Paradox', 'Paramis', 'SP06C2P5', 'Tudor', 'Daciana' (Table 3).

pH

Regarding pH, the results showed homogeneity is high enough and varying within a 3.25 and 4.43. Compared to the control varieties, 'Isadora' and 'Tudor' cvs. recorded the highest pH values (Table 3).

Titratable acidity

Organic acids together with sugars are the main soluble components of ripe fruits and have a major effect on taste, being responsible for acidity and aroma, at the same time, acidity is one of the main maturation indices that determine the date of harvesting of the fruits used either for direct consumption or for industrial processing (Neri et al., 2003; Crucirescu, 2022). With the approximation of the seeds of maturity, the fruit begins to ripen, and the concentration of acids decreases (Walker, 2011; Moscatello, 2012).

The most represented acid in the pear is malic acid, has the highest values at 'SP06C2P5' (0.53 %), 'Romcor' (0.50%). Of the analyzed varieties, 'Xenia' control cultivar had the lowest malic acid value 0.17%, the other control varieties had the content in malic acid 'Williams' (0.45 %) and (0.30%). In 2017, Butac & Militaru found values of malic acid for 'Monica' cv. of 0.25% and 0.30% for 'Isadora' cv. (Table 3).

Total polyphenols content

The content of phenolic compounds in fruits depends on intrinsic and extrinsic factors, such as the cultivar, part of fruit, agronomic practices, environmental conditions, maturity stage, and harvesting, in juices it also depends on the method of processing (Teixeira et al., 2023).

The polyphenolic content in pears is similar to that in apples (1654.8-5314.1 mg/kg DW) and quince (2609.50 mg/100 g DW) (Kolniak-Ostek et al., 2020; Górna´s et al., 2015; Teleszko et al., 2015). The results indicated that the varieties coming from *Pyrus serotina*, namely 'Isadora' and 'Tudor' have the highest content in polyphenols, respectively 4027.53 mg GAE/kg FW and 4000.00 mg GAE/kg FW, far exceeding the varieties taken as control (Table 4). Large values of the polyphenols content recorded at the following varieties: 'Triumf' (3114.49 mg GAE/kg FW), 'Paradox' (2788.41 mg GAE/kg FW), 'Cristal' (2679071 mg GAE/kg FW). On the contrary, the genotypes 'Paramis' and 'P20R41P30' showed lower contents, respectively 1167.27 mg GAE/kg FW and 1010.15 mg GAE/kg FW.

DW (%)

The fruit pears contain on average 79.19-87.23 % water, the rest is dry weight (DW). It consists of carbohydrates, proteins, lipids, minerals, organic acids, vitamins, phenolic compounds.

Table 4. The chemical properties of the fruit in the pear genotypes studied

Genotype	Total polyphenols content (mg GAE/kg FW)*	Water content (%)*	DW (%)*	Vitamin C (mg/100 g FW)*
Argessis	2178.26± 15.67[ef]	84.76± 0.25[ab]	15.24± 0.25[ef]	7.79± 0.20[bc]
Carpica	1879.71± 33.21[fgh]	83.51± 0.09[bcde]	16.49± 0.09[cde]	9.25± 0.47[a]
Cristal	2679.71± 488.63[d]	83.97± 0.25[bcd]	16.03± 0.25[cde]	7.55± 1.33[bc]
Daciana	2034.78± 4.35[fg]	85.79± 1.01[a]	14.21± 1.01[f]	3.49± 1.06[h]
Haydeea	2446.38± 354.19[de]	84.21± 0.73[bc]	15.45± 0.38e[f]	7.11± 0.60[bcd]
Isadora	4027.53± 33.21[a]	79.49± 0.25[f]	20.51± 0.25[a]	5.25± 0.14[fg]
Monica (Ct_1)	2820.29± 395.78[cd]	83.48± 0.40[bcde]	16.52± 0.40[cde]	10.31± 0.44[a]
P20R41P30	1010.15± 100.50[i]	85.76± 0.36[a]	14.24± 0.36[f]	8.05± 0.69[b]
Paradox	2788.41± 200.01[cd]	80.63± 0.08[f]	19.37± 0.08[a]	7.83± 0.94[bc]
Paramis	1167.27± 19.95[i]	82.21± 0.30[e]	17.79± 0.30[b]	6.22± 0.27[def]
Romcor	3315.94± 149.12[b]	82.72± 0.07[de]	17.28± 0.07[bc]	4.79± 0.34[g]
SP06C2P5	1594.2± 319.86[h]	84.23± 0.26[bc]	15.77± 0.26[de]	7.74± 0.38[bc]
Triumf	3114.49± 18.10[bc]	85.78± 2.39[a]	14.22± 2.39[f]	6.65± 0.36[cde]
Tudor	4000± 69.97[a]	84.12± 0.08[bc]	15.88± 0.08[de]	4.46± 0.27[gh]
Williams (Ct_3)	2475.36± 246.77[de]	83.00± 0.25[cde]	17.00± 0.25[bcd]	5.6± 1.34[efg]
Xenia (Ct_2)	1708.69± 23.01[gh]	83.51± 0.19[bcde]	16.49± 0.19[cde]	5.19± 0.32[fg]

*Values followed by the same letter are not statistically different according to DMRT ($P<0.05$).

Essentially, total dry matter reflects the quality of the fruit at harvest (Kader, 2002; Paraschiv & Nicola, 2023), the most relevant components being starch and soluble dry matter (Travers, 2013).

Highly significant differences, statistically assured by Duncan's multiple comparisons test, regarding DW were obtained between cultivars. This quality parameter was significantly higher in 'Isadora' and 'Paradox' varieties (20.51% and 19.37%, respectively). For the ˈDacianaˈ and ˈTriumfˈ varieties, the average recorded value of this indicator was only 14.21 % and 14.22 %, respectively (Table 4).

Vitamin C (mg/100 g FW)

A very important element of the quality of the pears is vitamin C. The content in vitamin C on pears is very low compared to apple. The results of vitamin C content of pears genotypes investigated in this study are presented in Table 4. These results show that there are statistically significant differences between the pear genotypes. ˈMonicaˈ control cv. gave the highest results in vitamin C content (10.31 mg/100 FW), followed by ˈCarpicaˈ (9.25 mg/100 FW). At the other varieties the value of the vitamin C content was between 4.46-8.05 mg/100 FW. The smallest value was registered with the ˈDacianaˈ variety, 3.49 mg/100 FW. According to Radulescu (1994) the contents of pears in vitamin C is between 2.31mg/100 FW on the ˈMeskiˈ cv. and 16.4 mg/100 FW at the ˈSanta Mariaˈ cv., which partially confirms our study (Table 4).

The color

The CieLa*b* color space is organized in the form of a cube. Axis L is executed from top to bottom. The maximum for L is 100, which represents the white color, and the minimum for L is zero, which represents black. Axes a* and b* have no specific numerical limits. The positive values to show the color red and the negative values show the green color. Positive values for b* show yellow color, and negative values blue color (Butac et al., 2012).

The color of the fruits is a quality commercial element, the colored fruits usually having a higher success on the market. The color of the fruits is a quality commercial element, the colored fruits usually having a higher success on the market. The pear peel contains chlorophyll (green) or carotenoid (yellow) pigments that give the color of the fruits. The ratio between these pigments influences the color, and can vary from dark green to yellow. Compared to apples where the heredity of the color is polygenic, in the background color it is determined monogenic, the allele for the yellow color is dominant to the alley for the green color. The red color is given by the anthocyanin pigment, the allele that determines this pigment is recessive to the allele that determines its lack, which is why many varieties have no color (Sestraș, 2004).

There were significant differences between cultivars influenced by the genetic factor. The lightness L* is ranging from 42.04 on ˈArgessisˈ to 66.91 on ˈTudorˈ. Compared to the values of the lightness at the control 'Williams' (L=62.24), three of the studied cultivars ('P20R41P30', 'Romcor' and 'Daciana' cvs.) were registered significantly higher values, the other two cultivar varieties 'Monica' and 'Xenia' were significantly lower values. As for axis a*, five of them have positive values 'Argessis', 'Carpica', 'Haydeea' and 'Triumf' which indicates the red color of the fruits. Regarding the axis b* the results obtained show that the genotypes obtained are yellow (Table 5).

Table 5. Color of the fruit pear-measured with the Konica Minolta colorimeter (Lab)

Genotype	L*	a*	b*
Argessis	42.04±4.13^{d}	7.43±5.41^{ab}	16.36±2.95^{de}
Carpica	50.62±4.91^{abcd}	0.56±3.45^{bcd}	15.3±11.4^{e}
Cristal	55.97±7.54^{abcd}	-10.34±4.51^{ef}	24.95±3.33^{abcd}
Daciana	63.43±3.24^{ab}	-16.17±1.29^{f}	29.53±0.71^{ab}
Haydeea	45.85±6.54^{cd}	14.62±7.04^{a}	19.12±3.96^{cde}
Isadora	60.23±6.81^{abc}	-12.41±2.63^{ef}	28.76±3.26^{ab}
Monica (Ct_1)	59.82±14.74^{abc}	-1.79±2.08^{bcde}	25.95±6.7^{abc}
P20R41P30	65.44±0.99^{ab}	-3.51±4.52^{cde}	26.7±2.11^{abc}
Paradox	61.71±1.42^{abc}	-9.01±1.61^{def}	27.39±1.27^{abc}
Paramis	60.34±4.93^{abc}	-9.01±4.85^{def}	27.17±0.61^{abc}
Romcor	63.15±3.05^{abc}	-10.00±3.16^{def}	28.75±0.89^{ab}
SP06C2P5	49.28±2.18^{bcd}	-2.2±2.2^{bcde}	23.47±0.58^{bcde}
Triumf	50.24±21.39^{abcd}	2.15±17.00^{bc}	27.11±10.36^{abc}
Tudor	66.91±6.19^{a}	-1.96±3.77^{bcde}	33.62±2.48^{a}
Williams (Ct_3)	62.24±2.58^{abc}	-3.11±1.12^{bcde}	29.07±0.77^{ab}
Xenia (Ct_2)	54.49±17.4^{abcd}	-8.96±2.33^{def}	22.9±5.72^{bcde}

*Values followed by the same letter are not statistically different according to DMRT (P<0.05).

CONCLUSIONS

At the genotypes of Romanian pears in most cases, higher results have been obtained than in the foreign varieties taken as control.

Of the studied genotypes were noted: 'Paramis' and 'Tudor' cvs. for weight and caliber; 'Argessis', 'Carpica', 'Daciana' cvs. for firmness; 'Haydeea', 'Isadora', 'Triumf' cvs. for soluble solids content (% Brix); 'Isadora' and 'Tudor' cvs. for pH and total polyphenols content (mg GAE/kg FW); 'Daciana' and

'Triumf' for water content (%); 'Isadora' and 'Paradox' for DW (%); 'Monica', 'Carpica', 'P20R41P30' for vitamin C (mg/100 g FW).

It takes a continuous concern to complete the germplasm fund and find better gene quality sources.

For the characters taken in the study there is a great variability of gene sources, offering the breeders numerous choices for hybridization.

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INFLUENCE OF SOIL AND CLIMATE CONDITIONS ON SOME QUALITATIVE INDICES IN SOME CULTIVARS OF *PRUNUS CERASUS*

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Abstract

The paper presents aspects related to the soil and climate conditions specific to Giarmata, Timiş County, Romania, and their influence on the main qualitative indices of five cultivars of sour cherry (Prunus cerasus): Mari timpurii, Târgu-Jiu 505, Oblacinska, Grecia 2, and Meteor. The research was carried out during 2021-2022 in a family-type plantation on a preluvosol soil type. The main indices studied were: tree vigour, fruit set degree, production, biometric elements, and fruit chemical composition. The results highlighted the following aspects: the diameter of the trunk was between 125.2-170.5 mm, the diameter of the crown was between 3.5-4.6 m per row and between 3.1-4.3 m between rows, the height of the trees was between 3.5-4.9 m, and the height of the crown was between 3.0-4.2 m. Fruit production ranged between 9.41 t/ha and 14.07 t/ha. Biometrics consisted of major diameter, minor diameter, tree height, tree size index and peduncle length. The chemical composition of the fruits was influenced by the soil and climate conditions, differing from one cultivar to another during the two study years.

Key words*: biometrics, cultivars, fruit chemical composition, Prunus cerasus, soil and climate conditions.*

INTRODUCTION

Most sour cherry cultivars originated from the species *Cerasus vulgaria* Mill. (common sour cherry) or from its hybrids with *Cerasus avium* (wild sour cherry) and *Cerasus fructicosa* Pall. (steppe sour cherry) (Mihuț, 2000; Ganopoulos et al., 2016; Rodrigues et al., 2008).

Sour cherry is a tree species of great economic importance due to the nutritional, technological, and commercial properties of its fruits, as well as the biological properties of the trees that can be used in various pedoclimatic conditions, from plain areas to submontane areas (Mihuț, 2002; Mihuț et al., 2022; Țărău et al., 2007; Radulov, 2007). In the western part of Romania, this species has adapted very well, thanks to favourable pedoclimatic conditions (Mihuț & Niță, 2018; Ianoș et al., 1997; OSPA Timișoara, 2021, 2022; Brady & Weil, 2003; Cresser, 1993). This species is found on about 6,000 ha, mostly in intensive system.

Compared to cherry, sour cherry has a greater ecological plasticity (Hillig & Iezzoni, 1988). It has low temperature requirements, and the biological threshold for bud swelling is 8ºC. Sour cherry is one of the most frost-resistant species among prunoids (-30ºC). It does well both in dry (400-500 mm of water annually) and humid regions (700-800 mm of water annually) (Parnaia et al., 1985). Having low light requirements, it can also be cultivated in north-west or north-east expositions (Mihuț & Drăgănescu, 2003).

Sour cherry, like the other fruit species, is extremely sensitive to both soil moisture excess an soil moisture lack. It bears fruit after about 3-4 years from planting, but the productivity is different, depending on the cultivar (Perez-Sanchez et al., 2008; Popescu et al., 1982).

In 1968, Olden & Nybom crossed *P. fruticosa* with *P. avium* and the resulting hybrid was very similar to *P. cerasus*, suggesting that they could be considered as the parents of the cultivated sour cherry (Beaver & Iezzoni, 1993; Hilling et al., 1988; Rodrigues et al., 2008).

Sour cherries are highly valued for consumption both fresh and in culinary preparations (Popescu et al., 1982). Research carried out over time has shown that the fruits have a beneficial effect on the human body, by regulating the acid-base balance, improving blood composition, in kidney, liver, and cardiovascular diseases, etc. (Nazari et al., 2012; Popescu, 1982). The energy value of sour cherries is about 63 kcal/100 g.

Sour cherry is commercially important, especially in the temperate climate zone (Toydemir et al., 2013). Furthermore, this culture helped develop new commercial cultivars of cherry, used as rootstocks, providing low vigour and resistant plants (Kappel et al., 2012). Many studies have been carried out to characterize genebank collections of *Cerasus* germplasm (Khadivi-Khub et al., 2012; Krahl et al., 1991; Nazari et al., 2012; Perez-Sanchez, 2010; Sánchez et al., 2008).

In many countries, the breeding material of sour cherry cultivars and traditional cultivars are compared, as they assess their performance under different climate conditions (Grafe & Schuster, 2014; Schuster et al., 2009, 2014; Siddiq et al., 2011).

Recently, some cultivars of Hungarian sour cherries were found to have functional properties that enhance the health benefits of the fruits (Papp et al., 2010; Veres et al., 2006).

On the world level, there is a tendency to increase the production of sour cherry fruits through the introduction into culture of cultivars with weaker vigour and with a higher potential (Parnaia, 1985).

FAO data (2021, 2022, 2023) show that Europe holds the largest share in world sour cherry production with 63.3%, followed by North America with 20.8%, Asia with 8.6%, and South America with about 0.6%. The major sour cherry producing countries are: Germany with 22.4%, Yugoslavia with 12.7%, Poland with 8%, and Hungary with 7.2%.

The annual production of sour cherries in our country is 6% of that of Europe, Romania ranging sixth among sour cherry producing countries (Mihuț, 2000).

The largest producer of sour cherries in Romania is Iasi County with 6,239 t, followed by the counties of Botoșani with 4,361 t, Bacău with 3,161 t, Argeș with 1,945 t, Buzău with 1,764 t, Cluj with 1,506 t, Dolj with 3,161 t, and Vâlcea with 741 t (Mihuț & Drăgănescu, 2003).

The assortment of sour cherries has continuously narrowed in recent decades, keeping a limited number of cultivars in culture, somewhat richer in Europe and poorer on other continents.

Currently, the promotion of some cultivars with small trees is being pursued to ease the work of tree care and fruit harvesting (Schuster et al., 2014). In general, the plain area of Banat, where the researched territory also falls, is part of Zone I of sour cherry distribution in Romania (Mihuț, 2000; Popescu et al., 1982).

MATERIALS AND METHODS

The researches were carried out on a family-type plantation located in the town of Giarmata (45°83' north latitude and 21°32' east longitude), Timiș County, during 2021-2022.

As part of the experiment, five cultivars of sour cherry with different periods of vegetation were researched:

- early cultivars: Mari timpurii, Târgu-Jiu 505, and Meteor;
- medium cultivars: G 2 (Grecia 2);
- late cultivars: Oblacinska.

1. The Mari timpurii cultivar is a very widespread cultivar in Romania, with medium vigour and good resistance to diseases and pests. Mocăneşti 16 is used as a pollinator.

The time of ripening of the fruits is in the middle of June.

2. The Târgu-Jiu cultivar, an autochthonous cultivar, is widespread in culture especially in the south of the country (Oltenia), which, however, has also adapted very well in Western Romania. The tree has medium vigour, it is resistant to frost and to the main sour cherry diseases: *Cocomyces hiemalis* and *Monilia*.

The ripening period is in the middle of June.

3. The Oblacinska cultivar comes from Serbia, being a self-fertile cultivar, resistant to frost and *Cocomyces* and sensitive to *Monilia.* Ripening period July 1-5.

4. The Grecia 2 (G2) cultivar, a cultivar originating in Grecia. The tree has medium vigour, and is resistant to frost and diseases.

5. The Meteor cultivar is a cultivar from the USA (the state of Minnesota) obtained in 1935 by hybridizing the Montmorency x Vladimirskaia x Subinka cultivars. It is a self-fertile cultivar, resistant to frost and cryptogamic diseases.

The ripening period is between July 12-15.

Location of the trial

The trees were planted in the spring of 2014, at 4 x 3 m, with a density of 883 trees/ha. The rootstock of the sour cherry was the frank sour cherry.

The variants of the trial are represented by five cultivars:
V1 – Mari timpurii;
V2 – Oblacinska;
V3 – Târgu-Jiu;
V4 – Meteor;
V5 – Grecia 2 (G2).
The maintenance works were those recommended by the technology in force. The formation cuts were made with few interventions, the crown being led in an improved vessel system and low bush. Phytosanitary treatments were done at the warning.
The data taken from the plantation were entered in the observation book and, based on them, the results presented in the paper were obtained.

Working Method
The progress of the fruiting phenophases was followed in the orchard, noting the moment of the beginning of each phenological phase.
The frost resistance of the flower buds was determined by the anatomical method, that is, by longitudinal sectioning of the flower buds and observing the tissues under a microscope.
Fruit production was determined by weighing the fruits per tree, then calculated for each cultivar. To calculate production per ha, it was multiplied by the density of 833 trees/ha.
Fruit biometrics was determined by detailed measurements regarding: the large diameter, the small diameter, the height, and the length of the peduncle. Within each cultivar, 50 fruits were measured, then averages were calculated for each cultivar.
Fruit acidity was determined by titration with NaOH 0.1 N, in the presence of phenolphthalein, expressed as a percentage (%) by multiplying g/kg by 0.067 malic acid equivalent.
The size index (Si) was calculated according to the formula:

$$Im = \frac{D + d + H}{3},$$

where: Im - Size index; D - large diameter; D - small diameter; H - fruit height.
% = (N x 4.25)/4-2.5,
where: N - number on the refractometer (in oBrix); 4.25 - juice density; 4 - stability coefficient; 2.5 - % of dry matter of an unsweetened nature (Scedei et al., 2021, 2023; Drăgunescu et al., 2021).
Total carbohydrate content was calculated based on soluble dry matter.
To characterize the climate conditions, the climatic data recorded and interpreted by the discipline of Agrometeorology, within the Faculty of Agriculture in Timișoara, were used (Mircov et al., 2022).

RESULTS AND DISCUSSIONS

1. Results Regarding the Pedological Conditions
The Commune of Giarmata is located on the south-eastern end of the Vinga plain, with a general northeast to southwest orientation. The Vinga plain has average altitudes between 100 and 150 m, very wide interfluves, sprinkled with depression areas, weak fragmentation, and less relief energy (Mircov et al., 2022)
From a geomorphological point of view, the town of Giarmata is in the Banato-Crişană Plain, part of the Western Plain.
From a hydrographic point of view, the researched territory is part of the Bega river basin, the Bega-Beregsău basin.
The territorial soil unit on which the experiment was carried out is of cambic chernozem, batigleyc, clay loam/clay loam type, on fine medium loessoid deposits (Munteanu & Florea, 2009; Posea, 2009).
The morphological properties of this soil indicate a moderate stage of development, characteristic of a relatively young soil, towards a soil with a certain degree of maturity (Mihuț & Niță, 2018). This soil has a slightly acidic to neutral reaction (pH of 6.4-6.8), a humus content of 3.6%, a degree of saturation in bases over 84%, and a water table at 2.5 m. Carbonates occur at more than 80 cm depth (Cca horizon), in amounts of 0.16% to 15%.
In conclusion, the soil on which the plantation is located has a medium fertility. To increase its fertility potential, agrotechnical and agrochemical measures are required to improve its physical and chemical properties.
From the wide range of soil types and subtypes in Banat, the sour cherry tree finds favourable conditions due to the favourable properties of this type of soil Mihuț et al., 2022.

2. Results Regarding Climate Conditions

Giarmata benefits from the same moderate temperate continental climate as most of Timiş County. From September to February, there were frequent intrusions of continental polar air masses coming from the east. The multiannual average temperature is 10.2ºC. The thermal characteristics of the hot season highlight the early onset and persistence of the western anticyclone, which makes the summer season start from May and continue until September. Periods of unstable weather are caused by the intermittent superimposition of colder north-westerly air masses over warm subtropical air masses. Multiannual average summer temperatures frequently exceed 20ºC.

The multiannual average temperature values, in the 5-10 cm layer, are higher by 2-4ºC, compared to those in the air.

Table 1 presents the temperatures recorded during the two years of research (2021 and 2022).

Table 1. Average monthly and annual temperatures recorded at the Timişoara Meteorological Station during 2021-2022 (Average monthly temperature ºC)

Year	Months												Mean (Sum)
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2021	-0.1	-2.0	6.7	10.9	15.7	20.1	23.6	20.5	18.3	13.1	5.7	2.6	135.1
2022	-0.9	2.9	7.2	10.8	16.9	19.7	21.1	21.2	20.2	11.3	2.8	1.6	129
Multi-annual Mean	-0.5	-2.45	6.95	10.85	16.3	19.9	22.35	20.85	19.25	12.2	4.25	2.1	132.05

The absolute minimum temperature was -27.2°C on February 16, 2021, the maximum winter temperature was 17°C, and the minimum summer temperature was 9°C.

The first frost date was October 23, 2021, and the last frost date was May 12.

The sum of the temperature degrees during the researched period was: 129ºC in 2022 and 135.1ºC in 2021.

The multiannual average of temperatures during the studied period was 132.05ºC.

Table 2 shows the average temperatures for the four seasons.

Table 2. Annual average at the Timişoara Meteorological Station in the period 2021-2022

Timişoara Meteorological Station	Annual mean during2021-2022			
	Spring	Summer	Fall	Winter
	12.02	21.35	11.32	0.83

The number of sunny days over the year was 29; average annual insolation fraction is 0.4; maximum monthly insolation fraction was in July, 0.80; minimum monthly insolation fraction, in January, was 12; fraction of insolation during the vegetation period (1.III – 31.X) was 0.52. The sum of the multiannual average of the researched period was 2,195.7 hours.

The average annual precipitation recorded in the period 2021-2022 was 630.6 mm according to the data presented in Table 3.

Table 3. Amount of precipitation (mm)

Year	Months												Mean (Sum)
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2021	18.1	27.7	52.2	45.5	34.9	49.8	33.8	34.5	41.0	33.2	60.8	70.5	502
2022	57.8	38.0	29.4	74.5	61.5	65.9	78.7	104.2	81.7	57.0	60.7	75.8	785.2
Multi-annual Mean	37.95	32.85	40.8	60.0	48.2	57.85	56.25	69.35	61.35	45.1	60.75	73.15	643.6

The total amount of precipitation for the period 2021-2022 was 502.0 mm in 2021, and 785.2 mm in 2022, with a multi-year average of 643.6 mm.

Regarding the relative humidity of the air, the data presented in Table 4 highlight the fact that, during the summer, there is a humidity deficit, which needs to be reduced by appropriate agrotechnical measures or supplemented by irrigation.

Table 4. Relative air humidity (%)

Year	Months												Mean
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2021	82	85	82	69	60	61	62	62	88	76	81	81	74.0
2022	85	80	73	66	70	74	67	69	82	80	85	87	75.7
Multi-annual Mean	83.5	82.5	77.5	67.5	65.0	67.5	64.5	65.5	85.0	78.0	83.0	83.0	74.85

The frequency and average speed of the wind in the main directions highlight the highest share of the north-west winds (21.2%) and of those from the west (15.6%), followed by those from the south (15.1%) and from the north (12.0%).

The annual average number of days with wind speeds greater than 11 m/s was 26.8, and of those with wind speeds greater than 16 m/s was 2.6. The period of calm characterised about 20.9% of the time, the average annual frequency of calm being 40.1%.

3. Results regarding the main qualitative indices

The data were statistically analyzed by the Duncan method, the IBM SPSS statistical software was used.

Tree vigour

The vigour of the trees is given by a complex of internal and external factors, namely: the hereditary characteristics of the cultivar, the rootstock, the favourable soil, and climate conditions and, finally, the agricultural techniques applied. Figure 1 and 2 shows the results of the measurements for the five cultivars studied.

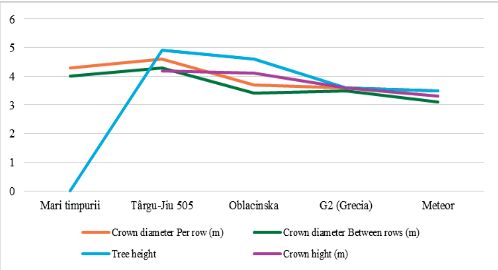

Figure 1. Crown diameter, Tree height and Crown height depending on variety

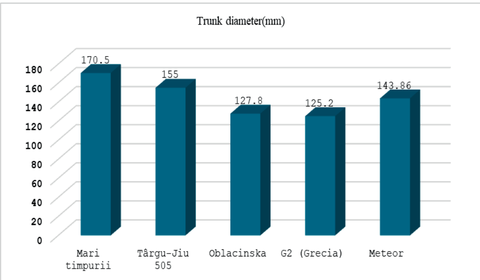

Figure 2. Trunk diameter according to variety

From the data in Figures 1 and 2, the diameter of the trunk is between 125.2 mm for the Meteor cultivar and 170.5 mm for the Mari timpurii cultivar, which confirms the fact that the rootstock used gives each cultivar a different vigour, but which is also influenced by the genetic characteristics of the respective cultivars, a fact that can also be observed following the performance of the other measurements. Thus, the diameter of the crown varies between 3.5 m per row and 3.1 m between rows for the Meteor cultivar and between 4.6 m per row and 4.3 m between rows for the Târgu-Jiu cultivar.

Fuit setting

In sour cherry, in the interval between the swelling of the buds and flowering, a series of biochemical processes and physiological transformations of an anatomical order take place inside the buds that lead to the formation of pollen grains, ovule and embryo sac. The formation of pollen grains takes place in the spring from the beginning of the swelling of the fruit buds and it lasts until the full debudding and the appearance of buds.

Flowering and setting of fruits, in sour cherry, takes place simultaneously with the leafing and is done based on reserve substances accumulated in the previous year.

For the partially self-fertile and self-sterile cultivars, it is necessary to plant them together with the pollinating cultivars.

Limiting ourselves only to the climate factor (temperature, precipitation, atmospheric humidity, and insolation) is directly related to the phenophases of development and fruiting, being largely responsible for the normal course of the annual cycle (Table 5).

Table 5. Degree of fruit setting (%)

No. crt.	Variety	2021	2022	Mediate 2021-2022
1.	Mari timpurii	25,91^{a}	28,60^{a}	27.25^{a}
2.	Târgu-Jiu 505	24,86^{a}	26,52^{a}	25.69^{a}
3.	Oblacinska	35,5^{ab}	35,8^{b}	35,65^{b}
4.	G2 (Grecia)	43,5^{b}	44,7^{c}	44,1^{b}
5.	Meteor	31^{ab}	33,5^{b}	32,25^{a}
Average		**32.15**	**33.8**	**32.98**

Means followed by the same letter do not differ statistically (Duncan test).

The research results presented in Table 5 show differences regarding this biological process. Thus, the weakest cultivar that sets the fruits (below 35%) is the Târgu-Jiu 505 cultivar, and the cultivars that set the fruits well (over 35%) are the Oblacinska, Grecia, and Meteor cultivars. In both research years, the Grecia cultivar set fruits the best, with 43.5% in the year 2021, respectively 44.7% in the year 2022. The weakest cultivar that set the fruits was the Târgu-Jiu cultivar, with 24.86% in 2021, respectively 26.52% in 2022.

The average fruit setting of cultivars was 30.01% in 2021 and 32.5% in 2022.

Sour cherry pollination is entomophilous, the pollen being difficult to release from the anthers; therefore, it is recommended to introduce beehives in the orchard.

Production

Fruit production was recorded per tree and then production per ha was calculated.

The average production per tree varied from one cultivar to another, as can be seen from Table 6.

Table 6. Fruit production (2021-2022)

Nr. crt.	The researched variety	2021		2022		Mediate 2021-2022
		kg/tree	kg/ha	kg/tree	kg/ha	
1.	Mari timpurii	$16,5^{c}$	$13744,5^{c}$	$17,3^{c}$	$14410,^{c}9$	14077,7
2.	Târgu-Jiu 505	$13,7^{b}$	$11412,1^{b}$	$12,5^{a}$	$10412,5^{a}$	11120,5
3.	Oblacinska	$14,2^{b}$	$11828,6^{b}$	14^{b}	11662^{b}	17493
4.	G2 (Grecia)	$13,8^{b}$	$11495,4^{b}$	$13,4^{b}$	$1162,2^{b}$	11328,8
5.	Meteor	$11,6^{a}$	$9662,8^{a}$	11^{a}	9163^{a}	9412,9
Mean of varieties		13.96	11,628.683	13.64	9,362.12	12,686.58

Means followed by the same letter do not differ statistically (Duncan test).

Sour cherry fruit production is between 11.0 kg/tree for the Meteor cultivar and 17.3 kg/tree for the Mari timpurii cultivar, productions that were recorded in 2022. The most productive cultivar was the Mari timpurii cultivar and the least productive, the Meteor cultivar.

The average of the cultivars over the two years was between 11.6 kg/tree in 2021 and 17.3 kg/tree in 2022. The average production over the two years was 12,686.58 kg/ha.

Fruits Biometrics

The size of the fruits, the intensity of the colour, and the length of the peduncle are elements that are of interest both for the recognition of the cultivars and for establishing their commercial and industrial processing value.

In general, above-medium to large-sized fruits are preferred, suitable for fresh consumption, and the cultivars with smaller fruits are mainly used for industrialization.

Thus, biometrics were made on more than 25 fruits of each cultivar. Following these measurements, the following were determined: the large diameter, the small diameter, and the height and thelength of the peduncle (Table 7).

From the data in Table 7, the fruits of the researched cultivars are of medium size. The large diameter being between 20.5 mm for the Mari timpurii cultivar and 17.7 mm for the Grecia 2 cultivar, and the small diameter between 19.8 mm for the Mari timpurii cultivar and 16.3 mm for the Oblacinska cultivar.

Table 7. Biometric elements of fruits (mm)

Nr. crt.	Cultivar	Diam. big mm	Diam. small mm	H. fruits mm	Size index	Long. peduncle	Quantities for consumption 1- consumption 2-industry
1.	Mari timpurii	$20,5^{b}$	$19,8^{b}$	19^{b}	$19,7^{b}$	$15,5^{a}$	1 2
2.	Târgu-Jiu 505	$19,7^{b}$	$17,4^{a}$	$16,5^{a}$	$17,8^{ab}$	$49,4^{c}$	1 2
3.	Oblacinska	$17,9^{a}$	$16,3^{a}$	$15,5^{a}$	$16,5^{a}$	$32,3^{b}$	2
4.	G2 (Grecia)	$17,7^{a}$	$16,5^{a}$	16^{a}	$16,7^{a}$	$39,5^{b}$	1 2
5.	Meteor	20^{b}	18^{a}	$17,5^{b}$	$18,5^{b}$	$35,5^{b}$	1 2
Cultivar Mean		19.16	17.6	16.9	17.84	34.44	-

Means followed by the same letter do not differ statistically (Duncan test).

The size index, which represents the size of the fruits, is between 19.7 mm for the Mari timpurii cultivar and 16.5 mm for the Oblacinska cultivar.

The length of the peduncle is between 49.4 mm for the Târgu-Jiu 505 cultivar and 15.5 mm for the Mari timpurii cultivar.

Chemical Composition of Fruits

The content of the fruits in various chemical substances differs from one cultivar to another, depending on the soil conditions, climate and applied agrotechnics.

From Table 8 on average over the two years of research, the dry matter is between 15.42 mg% for the Târgu-Jiu cultivar and 11.50 mg% for the Mari timpurii cultivar, with an average of 14.54 mg% in the year 2021, and 14.46 mg% in 2022, respectively.

Table 8. Chemical composition of sour cherry fruits (2021-2022)

Cultivar	Year	Dry matter (%)		Total Sugars (%)		Acidity		Sugars / Acidity
		Mean/year	Mean 2021-2022	Mean/year	Mean 2021-2022	Mean/year	Mean 2021-2022	Mean 2021-2022
Mari timpurii	2021	11.50	13.05	9.72	11.36	1.02	1.13	10.05
	2022	14.60		13.01		1.25		
Târgu-Jiu 505	2021	15.42	14.52	13.88	11.93	1.34	1.41	8.46
	2022	13.63		11.98		1.48		
Oblacinska	2021	14.92	14.71	14.35	13.63	1.53	1.64	8.31
	2022	14.50		12.91		1.75		
G2 (Grecia)	2021	14.32	14.71	12.71	13.12	1.40	1.32	9.94
	2022	15.10		13.54		1.25		
Meteor	2021	14.72	14.61	13.14	13.03	1.30	1.25	10.42
	2022	14.50		12.91		1.20		
Cultivar Mean	2021	14.54	14.56	12.95	12.90	1.25	1.35	9.56
	2022	14.46		12.86		1.45		

The content in total carbohydrates calculated based on soluble dry matter is between 11.36% in the Mari timpurii cultivar and 13.63% in the Oblacinska cultivar, the average of the cultivars being 14.59%.
The carbohydrate/acidity ratio is between 10.42% for the Meteor cultivar, 10.05% for the Mari timpurii cultivar, 8.46% for the Târgu-Jiu% cultivar, and 9.94% for the Grecia 2 cultivar, the average of the investigated cultivars being 9.56%. Cultivars with a carbohydrate/acidity ratio below 10% are less suitable for fresh consumption, as they have high acidity.
Correlating this ratio with the size and quality of the pulp, it was found that the best fruits for fresh consumption are the cultivars Mari timpurii and Târgu-Jiu 505.

CONCLUSIONS

Located in the central-northern area of Timiş county (45°83' north latitude and 21°32' east longitude), Giarmata is 11 km from Timişoara and 1.3 km from Timişoara International Airport.
From a geomorphological point of view, the area of the commune falls within the Banato-Crişană Plain, part of the Western Plain of Romania, the eastern extremity of the Tisa Plain.
The climate is moderately temperate-continental with warm summers and mild winters due to both the influences of oceanic (from the W) and Mediterranean (from the S and SW) air masses. The thermal characteristics of the hot season highlight the early onset and persistence of the western anticyclone, which makes the summer season start in May and continue until September. Periods of unstable weather are caused by the intermittent superimposition of colder north-westerly air masses over warm subtropical air masses. Multiannual average summer temperatures frequently exceed 20ºC.
The date of the first frost recorded during this period was on October 23, 2021. The date of the last frost was on May 12, 2021.
The sum of the temperature degrees during the researched period was 129ºC in 2022 and 135.1ºC in 2021.
The multi-year average of temperatures during 2021-2022 was 132.05ºC.
Regarding precipitation, the annual average was 649 l/m^2, but it is unevenly distributed. Abundant precipitations and excess moisture in the spring period, in the crop-open field system prevent seasonal work from being carried out in good conditions and at the same time hinder the processes of germination and emergence, at the same time favouring phytopathogenic attack, especially in cucumbers.
The prevailing winds are those from the northwest sector, determined by the Azores Anticyclone, with an average speed of 3.4-3.5 m/s. It is a warm and humid wind that brings the precipitations of May and June. Throughout the year, the Austrul blowing from the southwest, from the Adriatic Sea, is felt. The predominant air movement at high altitude is from west to east.
Winds blow from the north at a rate of 15.7%, from the east around 15.6%, and from the south with a frequency of 20.6%.
The wind intensity is low, and the monthly average, according to the Beaufort scale, is 2.5 m/second.
The annual average number of days with speeds greater than 11 m/s was 26.8, and with speeds greater than 16 m/s was 2.6. The calm period occupies about 20.9% of the time.
The climatic conditions and the soil in the plantation located in Giarmata commune are quite favourable for sour cherry culture.
The limiting factors that slow down the biological and physiological processes are the uneven distribution of precipitation during the year, the soil with a high clay content and the climate accidents represented by late spring frosts.
However, sour cherry cultivars have proven to be quite resistant to these conditions compared to other fruit tree species.
Regarding the vigour of the trees in the researched cultivars, they are divided into three groups:
- High vigour: Timpurii de Cluj, Mari timpurii;
- Medium vigour: Târgu-Jiu 505, Oblacinska;
- Low vigour: Meteor.

Fruit setting varies greatly from one cultivar to another. The cultivars Meteor and Gloria stand out for their good bonding.

The cultivar with a low fruit setting percentage is Târgu-Jiu 505.

Significant differences are noted between cultivars in terms of fruit production. Cultivars with good production are: Mari timpurii, Oblacinska, and Meteor.

The size of the fruit differs depending on the cultivar. It can be said that the researched cultivars have medium-sized fruits, except for the Mari timpurii cultivar, which has a large fruit.

The physico-mechanical analysis highlighted cultivars with a high proportion of pulp: Oblacinska, Mari timpurii.

In the chemical composition of the fruits, a carbohydrate/acidity ratio of over 10% was noted in the Mari timpurii and Meteor cultivars and under 10% in the Oblacinska cultivar.

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DAMAGE BY *DROSOPHILA SUZUKII* (MATSUMURA) FRUITS OF BLACKCURRANT CULTIVAR `TITANIA`

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Abstract

Drosophila suzukii (Matsumura) is a particularly dangerous species of tree fruit and berry fruit species in Europe and all over the world. It was first reported in 2014 in the regions of Blagoevgrad, Kyustendil, and Plovdiv in Bulgaria. Its harmful activity directly threatens the yield and in some years it can compromise the harvest. The present study aims to monitor the population density and fruit damage to blackcurrant fruits of the 'Titania' variety. The studies were conducted in the period 2019-2020 at the Research Institute of Mountain Stockbreeding and Agriculture of Troyan. Insect traps were used for monitoring, which were placed at the beginning of fruit ripening. As a result of the conducted observations, it was found that the spotted wing drosophila appeared at the beginning of fruit ripening, and its peak was reported during large-scale fruit ripening.

Key words: *Drosophila suzukii, damages, insect traps, blackcurrant.*

INTRODUCTION

Insects with broad food specialization cause significant economic losses in agriculture (Oerke, 2006). One such species is *Drosophila suzukii* (Matsumura), which attacks plants from various botanical families but has a particular preference for thin-skinned fruits such as cherries, sour cherries, plums, etc. (Baroffio and Fisher, 2011; Aspen et al., 2015).

According to Olazcuaga et al. (2019) Olazcuaga et al. (2023) females prefer to lay their eggs on fruits (blackberries, cherries, blackcurrants) rich in phosphorus. Phosphorus is present as inorganic or organic phosphate in fruits. A positive correlation was found between oviposition and phosphorus content in fruits. Phosphorus is important for the development and reproduction of female insects, including some species of the genus *Drosophila* (Bergwitz, 2012; King & Wilson, 1955; Markow et al., 1999). According to Markow et al., (1999), females actively seek phosphorus-rich foods because they use phosphorus for RNA transcription during oogenesis.

The homeland of *D. suzukii* is considered to be Southeast Asia (Bolda et al., 2010), where it was first found in 1916 on cherries in Japan by Kanzawa. Later in 1931, the species was described by Matsumura (Kanzawa, 1939).

D. suzukii was recorded in Europe and the USA almost simultaneously in 2008 (Cini et al., 2012). In the USA, damages of this species were first detected in September 2008 in raspberry plantations in Santa Cruz, California. At the end of 2009, the species was registered in many districts of the State of California, as well as other states of the USA such as Florida, Oregon, and Washington, as well as in the province of British Columbia in Canada (ERRO, 2010).

In Bulgaria, *D. suzukii* was detected for the first time in 2014 on the territory of three regions - Blagoevgrad, Plovdiv, and Kyustendil, in plantations with plums, cherries, peaches, and apples (Laginova and Ivanova, 2015). At first, it was discovered in a cherry orchard in Blagoevgrad, and in autumn in a plum plantation with the Stanley variety in the village of Tarnovlag. In the Plovdiv region (the village of Voyvodinovo and the village of Kalekovets), it is registered in apple and peach orchards, and in the fruit and vegetable market in the village of Parvenets. On the Black Sea coast in Varna, *D. suzukii* was found in a warehouse for fruit imported from Turkey, Greece, and Poland (Laginova and Ivanova, 2015), and in 2018, for the first time, flies were caught in the city of Burgas in fig plantations. In the same year, *D. suzukii* was also discovered in the village of Trun (Pernik region) in a nursery of raspberry mother plants, and in the village of Brashlen (Ruse region) in October 2018 in raspberry plantations.

D. suzukii prefers varieties with red fruits or late-ripening berries, with no firm fruit skin (Ioriatti et al., 2018).

Lee et al. (2011) found that *D. suzukii* has different preferences between individual varieties, as well as among varieties of the same species.

This study aimed to observe the population dynamics of *Drosophila suzukii* in blackcurrant plantations.

MATERIALS AND METHODS

The studies were conducted in the period 2019-2020 at the Research Institute of Mountain Stockbreeding and Agriculture of Troyan in blackcurrant plantations with 'Titania' variety.

The 'Titania' variety has large berries that reach a weight of 3.5 g and a high sugar content. The fruit skin of the berries is tough.

The monitoring of *Drosophila suzukii* was conducted with insect traps. For this purpose, traps were prepared, using plastic containers in the upper part, on which several holes with a diameter of 2-3 mm were made. Suzukii Trap nutritional mixture of the Spanish company Bioiberica and a classic mixture (red wine and apple cider vinegar in a ratio of 3:2) were poured into them. The traps were placed in the middle of the rows in early May when berries began to ripen. The mixture was renewed at each report. The collected material was placed in polyethylene bags and analyzed in a laboratory.

Damages on berries by *D. suzukii* were determined by visual observations on 50 randomly placed plants.

The data were processed statistically (Statistika v.7) and visualized in graphs.

RESULTS AND DISCUSSIONS

The appearance of *D. suzukii* in blackcurrant plantations is related to the ripening of the fruits because the larvae develop only in fruits with a minimum sugar content of about 14%, and average daily temperatures above 10ºC favor the development and multiplication of the species (Kanzawa, 1939).

The first adults of *D. suzukii* in the 'Titania' variety in 2019 were recorded in the second ten days of May (one fly in the classic trap and three flies in the Suzukii Trap, Figure 1). During this period, the average daytime temperatures were 14.8ºC, and the maximum reached 21.8ºC (Table 1).

With the warming of the weather, a period of increase in the number of pests in both types of traps began, which was directly related to the ripening of the fruits. During the first ten days of June, the fruits ripened to a large extent, and the pest significantly increased its number (24 flies in the classic trap and 45 flies in the Suzukii Trap). During this period, the soluble dry matter content of the fruit in terms of Brix was 12%. The density of flies in the traps decreased due to fruit harvesting.

Table 1. Agroclimatic characteristics for the area of Troyan

2019	Average T^{0}C	Min T^{0}C	Max T^{0}C	RH %	Rainfall mm
May	14.8	8	21.8	73	82.4
June	19.9	13.6	26.6	79	234.6
July	20.2	13	27.8	75	106.7
2020	**Average T^{0}C**	**Min T^{0}C**	**Max T^{0}C**	**RH %**	**Rainfall mm**
May	14.7	8.6	20.6	78	63.8
June	17.8	12.6	24.6	81	129
July	20.4	27.1	13.5	78	75.4

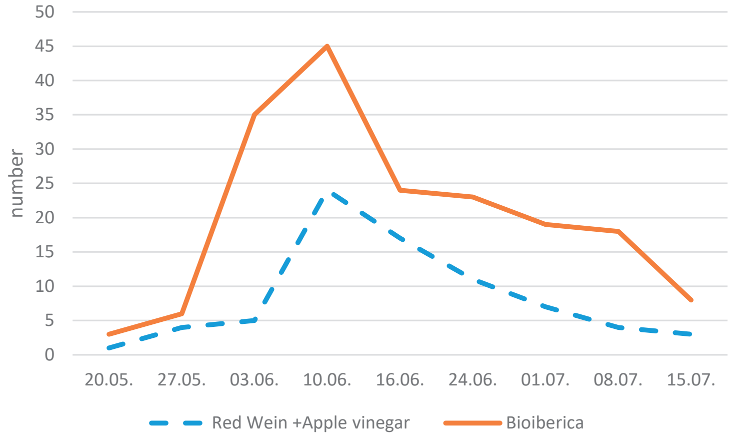

Figure1. Population dynamics of *D. suzukii* in blackcurrant variety 'Titania' in 2019 in the area of city Troyan

The first adults of *D. suzukii* were registered again in the second ten days of May 2020 (2 flies in the classic trap and 2 flies in the Suzukii Trap, Figure 1).
As the temperatures rose and the fruits ripened, their number increased in both types of traps. A peak in the multiplication of the species was recorded in the second ten days of June (44 flies in the classic trap and 55 flies in the Suzukii Trap).
During this period, the soluble dry matter content of the fruit in terms of Brix was 11%. As the fruits were harvested, the density of flies in the traps decreased.

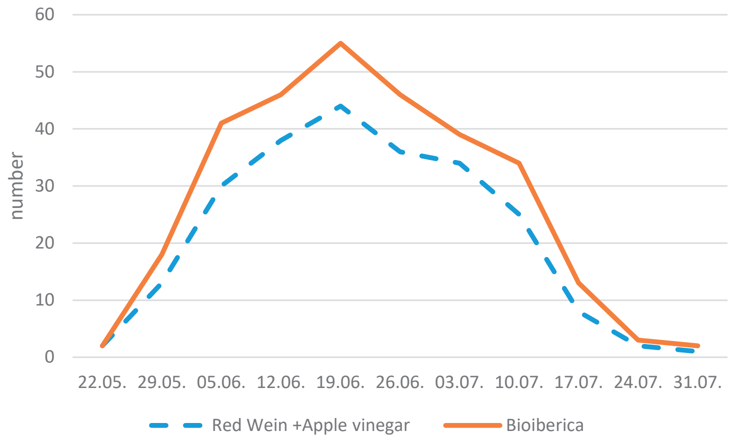

Figure 2. Population dynamics of *D. suzukii* in blackcurrant variety 'Titania' in 2020 in the area of city Troyan

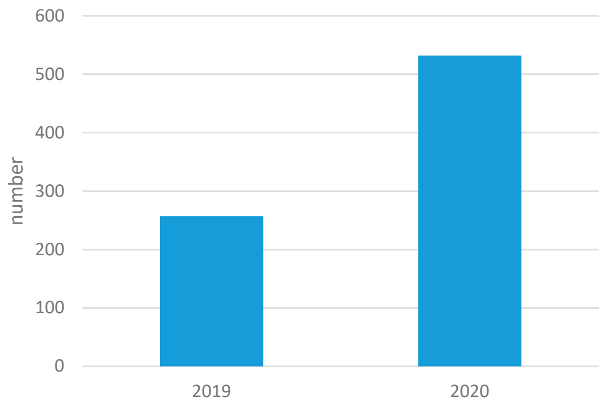

Figure 3. Caught adults of *D. suzukii* in 2019 and 2020 in 'Titania' blackcurrant plantations

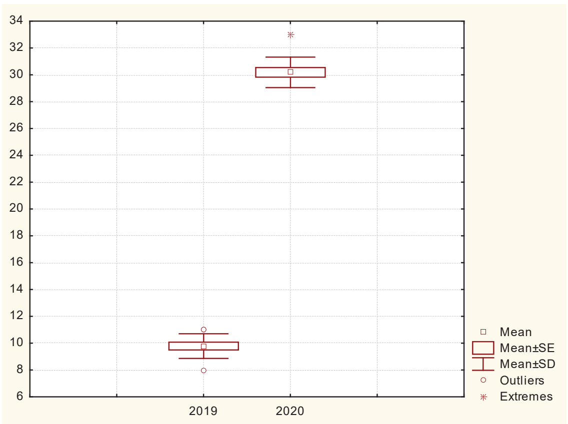

Figure 4. Detected damages on the fruits of the blackcurrant variety 'Titania' (%)

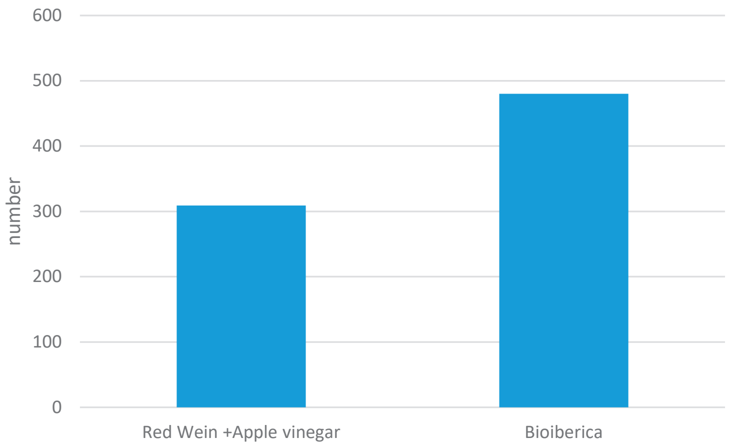

Figure 5. Caught adults of *D. suzukii* in the traps in 2019 and 2020

Damages to the fruits were also found during the inspections. In 2019, *D. suzukii* was registered in a lower number (257 flies) (Figure 3), as a result of which 10±0.01% damaged fruits were reported. In 2020, the environmental conditions favored the development of the species, 532 flies were recorded. 30±0.01 % damaged fruits were found (Figure 4).
During the research period, 480 flies were caught with the Suzukii Trap, and 309 flies with the classic trap. This can be explained by the higher pH in the "Bioiberica" nutrient mixture (Figure 5).

CONCLUSIONS

The following conclusions can be drawn as a result of the conducted studies:
The first adults of *D. suzukii* in the food traps of blackcurrant variety Titania were found in the second ten days of May (May 20-22), at that period the fruits begin to ripen for the Troyan area.
The highest number of *D. suzukii* flies was found during the large-scale fruit ripening when was registered the highest content of soluble dry matter according to Brix (12%).
In 2019, 257 flies were caught in the food traps, as a result of which the damaged fruit was 10 ± 0.01%; and in 2020, 532 flies were caught, and respectively 30 ± 0.01% of the fruit was damaged.
480 flies were caught in the Suzukii Trap, and 309 flies in the classic trap. This can be explained by the higher pH of the "Bioiberica" nutrient mixture.

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USE OF ECOLOGICAL METHODS TO CONTROL PATHOGENS AND PESTS IN APRICOT THE SOUTHERN AREA OF ROMANIA

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Abstract

The southeastern part of Romania is the area that offers the most favorable ecoclimatic conditions for apricot cultivation. The territory of the RSFG Constanta is influenced, from a climatic point of view, both by the Black Sea as well as the Danube, which means that spring arrives later summers are droughty and autumns are long and warm. The dynamics of the population of harmful microlepidoptera was realized with the help of AtraLIN, AtraMOL, AtraNUB pheromone traps. Trapping by specific sex pheromones initiated in 2020-2022 in an apricot plantation at the Research Station for Fruit Growing (RSFG), to monitor three pests, peach twig borer (Anarsia lineatella), oriental fruit moth (Cydia molesta) and (Hedyia nubiferana). The results of the three-year study showed that in 'Elmar' cultivar The amount of catches was higher for Anarsia lineatella (AtraLIN)in the Olimp cultivar, 341 butterflies in the year 2022 and the lowest amount of catches was for Hedyia nubiferana (AtraNUB), 267 butterflies in the year 2022.

Key words: *Prunus Armeniaca, symptoms, variety sensitivity monitoring, feromonal traps, ecological products.*

INTRODUCTION

The apricot tree culture is widespread in Europe, Asia, America and Oceania; almost 40% of the global production of 3,473,710 tons obtained in 2008 was produced in Europe, followed by Asia with 32%. The largest producer in the world is Turkey (528,000 tons), other large producers being Spain (159,000 tons), the USA (81,000 tons), Italy (212,000 tons), France (180,000 tons), Greece (68,500 tons) and so on. In Romania the apricot tree production in 2008 was of 32,100 tons (Stanica F. et al., 2011). Cytospora was first introduced by Ehrenberg (1818), which is one of the most important pathogenic fungi of hardwoods and coniferous trees in the world (Adams et al., 2005; Fan et al., 2020). About 150 species epithets of Cytospora are associated with dieback and stem canker on over 130 species of woody hosts (Adams et al., 2005; Fan et al., 2020). Among those who have previously dealt with the genetic resistance to diseases and pests în Romania are: Trandafirescu et al. (1989, 2005, 2006), Balan, et al 2008 and the creating of apricot tree cultivars Topor et al. (1997; 2006; 2007). For this reason, cultivating new cultivars that are resistant to the attack of these pathogens is the most important method to both prevent and control such attacks. Many researchers have recorded data concerning the reproduction of sources that are resistant to the attack of pathogens, such as: Crossa (1969), Keil (1976), Pascal et al. (1994), Trandafirescu (1989, 2006). Apricot breeding has been conducted by many researchers from the entire world where apricot tree finds good conditions to grow. Researchers from Italy (Penonne, 1999; Nicotra et al., 2006; Guerriero et al., 2006), France (Audergon et al., 1999), Spain (Egea et al., 1999), Greece (Syrgianidis et al., 1999), Bulgaria (Tsoneva, 1999), New Zealand (Hofstee, 1999), Slovak Republic (Benedicova, 2006) and many others have their own apricot breeding programs. In Romania, the apricot breeding program started in 1952, being under the leadership of Cociu (2006).

MATERIALS AND METHODS

The experimental plot is situated within the Research Station for Fruit Growing (RSFG) Constanta, with its headquarters in the village of Valu lui Traian, Constanta county, Dobrogea region, Romania. The geographical coordinates are: 44°10’ North, 28°29’ East, 70-72 m altitude. During the period 2020-2022, 2 apricot tree cultivars 'Elmar' and 'Olimp', were studied, organised in a demonstrative plot that was created in 2011. The plot has 20 trees per row, with a planting distance of 4/4 m (625 trees/ha), with the canopy shape a vase and the parent stock a wild apricot tree. The system used for the soil management system was with cultivated strips both between the rows as well as in the row. The soil is a calcareous chernozem (CZka), with a loamy texture and a high, alkaline pH (8.2) in its entire profile. All in all, the climatic conditions were favourable to the growth and fructification of the apricot trees.

'Elmar'- cultivar was obtained at R.S.F.G Constanta - Valu lui Traian, in 2008.

The flowering period is early, abundant; fruit ripening period: early (June 17-23); the cultivar is self-fertile, does not require pollinators; tolerant to the main specific apricot diseases, free of viruses. The fruit has an oval shape; the average weight is 45-55 g; skin: medium orange. It ensures benefits per hectare as a result of the high productivity of the fruit quality (attractiveness, size, taste, aroma) (Figure 1).

'Olimp' - cultivar was obtained at R.S.F.G Baneasa - Bucharest. The tree has medium vigor, reverse-conical crown, bears fruit predominantly on bunch branches (may bunches), late. The cultivar is partially self-fertile. The fruit is spherical - ovoid in shape, 65-75 g with an orange epidermis, with a little red on the sunny side. Ripening period: August 10-12 with disease and frost resistance (Figure 2).

Observations were carried out concerning the behaviour of two apricot tree cultivars towards the attack of the main pathogen agents: *Stigmina carpophila*, *Cytospora cincta* and *Monilinia laxa*. These observations were focused on the evolution of the diseases on the leaves, fruit and shoots following. From a technological point of view, 8-10 treatments with insecticides and fungicides have been administered each year in the experimental plots, so as to protect against diseases and pests. The system used for the soil management system was cultivated strips both between the rows as well as in the row.

Figure 1. 'Elmar' cultivar

Figure 2. 'Olimp' cultivar

The occurrence of the disease *(Monilinia laxa)* at the apricot tree is highly influenced by the atmospheric conditions: at a temperature of 4-6°C accompanied by precipitations, the disease is triggered within 48 hours, while at a temperature of 10°C, the disease is evident within 18 hours (Trandafirescu and Teodorescu, 2006). The behaviour of apricot tree cultivars towards the attack of the pathogen agents - 1) *Stigmina carpophila* (Lév.) M.B. Ellis, 2) *Cytospora cincta* Sacc and 3) *Monilinia laxa* (Aderhol et Ruhl.) - was studied under conditions of natural infections, according to the test created by Crossa Raynaud (1968).

The evaluation technique consisted in writing down the frequency of the attacked organs and the intensity with which the symptoms manifested themselves and these two aspects were utilised in assessing the behaviour of the cultivars.

The field observations were centred on the calculation of the pathogens' frequency (F %) and intensity (I) on different tree organs such as: leaves, flowers, shoots, branches and fruits. For the intensity of the diseases marks were granted on a scale from 0 to 4 (Table 1). Depending on the frequency and intensity of the disease, the studied cultivars and hybrids were categorised into 4 classes and 8 groups of resistance according to the following scale.

Table 1. Cultivar Repartition into Classes and Groups of Resistance

Resistance class	Resistance group	Frequency (F%)	Intensity (I%)
1= tolerant (T)	1	0	0
2= medium resistance (MR)	2	0.1-11.0	+
	3	11.1-25.0	+
3= sensitive (S)	4	25.1-34.0	++
	5	34.1-50.0	++
4= very sensitive (VS)	6	50.1-59.0	+++
	7	59.1-75.0	+++
	8	75.1-100	++++

WA = cultivars without attack (F%= 0 and I= 0); T = tolerant cultivars (F%= 0.1-5% and I= +); WeA = weakly attacked cultivars (F%= 5.1% - 10% and I= +); MA = moderately resistant cultivars (F%= 10.1% - 25% and I= +); S= sensitive cultivars (F%= 25.1 – 50% and I= ++++); VS = highly sensitive cultivars (F%= 50.1% - 100%, I= ++++)

The monitoring of the dynamics of the population of harmful microlepidoptera is carried out with the help of AtraLIN, AtraMOL, AtraNUB pheromone traps, on the 'Elmar' and 'Olimp' apricot cultivars, for the monitoring of harmful insects, *Anarsia lineatella, Cydia molesta* and *Hedya nubiferana* before the start of the flight in the experimental lots at the RDSFG Constanta, during three years 2020, 2021 and 2022. The synthetic sexual pheromones were procured from the Cluj-Napoca Chemistry Institute, http://iccrr.institute.ubbcluj.ro/contact.html.
The control of the traps was carried out weekly, the captured butterflies being recorded and removed so as not to influence the subsequent observations. The traps were installed in the crown of the trees at a height of 1.5 m, in May. The control of the traps was carried out weekly, the captured butterflies being recorded and removed so as not to influence the subsequent observations. The replacement of pheromone capsules was ensured every 4 weeks, respecting the distance of at least 50 m between traps. A pheromone-soaked bait is placed in the sticky tape trap. Spreading in the environment, it attracts the males into the trap. The butterflies stick to the special glue, which has no smell and no harmful substances added. Pheromones are allowed for wide use, they do not pollute the environment and are harmless to humans and animals. The catches recorded in each trap were entered in the tables, and based on the butterflies caught at each observation, the flight curve was made, in order to more easily follow the evolution of the population of harmful microlepidoptera. The obtained data highlight the beginning, the end and the maximum flight of each generation, elements necessary to establish the optimal moments of treatment.

RESULTS AND DISCUSSIONS

Table 2 presents the relative sensitivity of the two cultivars from the demonstrative plot created within the laboratory responsible with improving the apricot tree concerning the attack of the pathogens: *Stigmina carpophila* (Lév.) M.B. Ellis, *Cytospora cincta* Sacc, *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey under natural conditions of infection. The analysis of the data in this table highlights a variation in the apricot tree cultivars behaviour towards a pathogen or another. The observations that were carried out under conditions of natural infection with *Stigmina carpophila* (Lév.) M.B. Ellis for the two apricot tree cultivars displayed the different degrees of resistance.
'Elmar' cultivar was Tolerant (T) in the studied years 2020, 2021 and 2022 towards *Stigmina carpophila* (Lév.) M.B. Ellis. 'Olimp' cultivar displayed a Medium Rezistance (MR) in the studied years 2020, 2021 and 2022.
'Elmar' cultivar was Tolerant (T) in the studied years 2020, 2021 and 2022 towards *Cytospora cincta.* The 'Olimp' cultivar displayed a Medium Rezistance (MR) towards *Cytospora cincta* in the studied years 2020, 2010 and 2022.
During the study period 2020-2022 both 'Elmar' and 'Olimp' manifested a good resistance towards *Monilinia laxa*, being basically in the class Tolerant (T) and mean resistance (MR) to *Monilinia fructigena*.
That is why fruit-growing practices on the one hand and especially the RSFG Constanta programme for improving the apricot tree on the other highlighted as main objective for researches the promoting of cultivars and the identification possible genitors with genetic resistance towards the attack of the pathogens *Stigmina carpophila (Lév.) M.B. Ellis, Cytospora cincta* Sacc, *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey.

Table 2. The behaviour of apricot tree cultivars towards the attack of the main pathogens in the period 2020, 2021 and 2022

No.	Cultivar	Year	Attack intensity (notes)			
			Stigmina carpophila	***Cytospora cincta***	***Monilinia laxa***	***Monilinia fructigena***
1.	Elmar	2020	T	T	T	MR
		2021	T	T	T	MR
		2022	T	T	T	MR
2.	Olimp	2020	MR	MR	T	MR
		2021	MR	MR	T	MR
		2022	MR	MR	T	MR

The monitoring of the dynamics of the population of harmful microlepidoptera was carried out with the help of AtraLIN, Atra MOL, AtraNUB pheromone traps, in the apricot varieties 'Elmar' and 'Olimp', during 1.05-8.09.2020-2022 period.

In the apricot orchard from the experience, the attack with *Anarsia lineatella* was monitored and the flight dynamics and the total catches for the period May- September 2020 were realized.

It is observed that the maximum of the flight curves, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees at 'Elmar' variety was registered in the third decade of June 2020, 23 butterflies/trap/week (Figure 3).

In the experimental apricot orchard, the attack of *Cydia molesta* was monitored and the flight dynamics and the total catches were realized for the period May-September 2020.

It is observed that the maximum of the flight curve, determined using the atraMOL pheromone trap for *Cydia molesta*, was recorded in the secand decade of June on the 'Elmar' variety, 35 butterflies/trap/week (Figure 4).

In the experimental apricot orchard, the attack of the green bud moth *(Hedyia nubiferana)*, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the third decade of June 2020, 37 butterflies/trap/week (Figure 5).

From the analysis of Figure 6, it can be seen that the maximum value of the amount of catches, 255 male butterflies/trap/season, was recorded for the bud moth (*Hedya nubiferana*), followed by the peach moth (*Cydia molesta*) with an average amount of catches of 227 male butterflies/trap/season. From the same figure, the minimum level of the sum of the captures of the peach shoot moth (*Anarsia lineatella*) can be noted, namely 208 male butterflies/trap/season.

The monitoring continued also in the year of 2021. In the apricot experimental orchard, the attack by *Anarsia lineatella* was monitored and the flight dynamics and the total catches for the period May- September 2021 were realized.

It was observed that the maximum of the flight curve, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees for *Anarsia lineatella* at variety 'Elmar' was registered in the second decade of June 2021, 27 butterflies/trap/week (Figure 7).

In the apricot orchard where the experiment was set up, the attack with *Cydia molesta* was monitored and the flight dynamics and the total catches were made for the period May-September 2021. It is observed that the maximum of the flight curve, determined with the aid of the atraMOL pheromone trap for *Cydia molesta*, was recorded in the third decade of June at the 'Elmar' variety, 25 butterflies/trap/week (Figure 8). In the apricot orchard, the attack of the green bud moth (*Hedyia nubiferana*), a species with only one generation per year, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the third decade of June 2021, 24 butterflies/trap/week (Figure 9). From the analysis of figure 10, it can be seen that the maximum value of the amount of catches, 192 male butterflies/trap/season, was recorded for the bud moth (*Cydia molesta*), followed by the peach moth *(Hedya nubiferana)* with an average amount of catches of 183 male butterflies/trap/season. From the same figure, the minimum level of the sum of the captures of the peach shoot moth (*Anarsia lineatella*) can be noted, namely 178 male butterflies/trap/season.

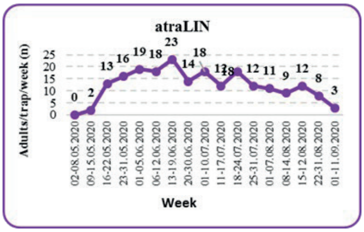

Figure 3. *Anarsia lineatelala* flight dynamic established with atraLIN at RSFG Constanta, 2020, 'Elmar' apricot cultivar

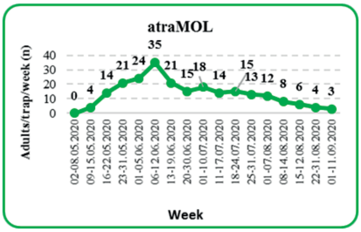

Figure 4. *Cydia molesta* flight dynamic, established with atraMOL pheromone trap at RSFG Constanta, 2020 'Elmar' apricot cultivar

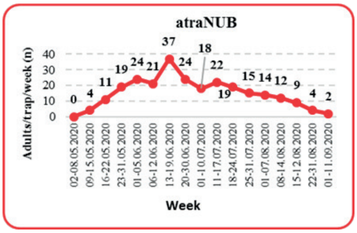

Figure 5. *Hedya nubiferana* flight dynamic established with atraNUB feromone traps at RSFG Constanta, 2020, 'Elmar' apricot cultivar

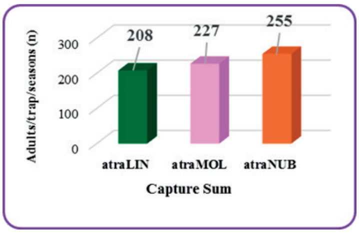

Figure 6. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2020, 'Elmar ' apricot cultivar

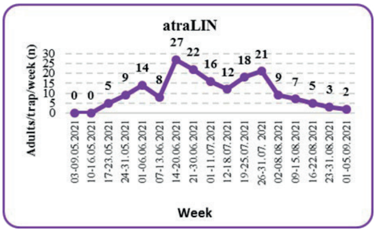

Figure 7. *Anarsia lineatelala* flight dynamic established with atraLIN pheromone traps at RSFG Constanta, 2021, 'Elmar' apricot cultivar

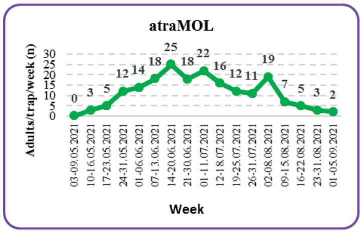

Figure 8. *Cydia molesta* flight dynamic, established with atraMOL pheromone traps at RSFG Constanta, 2021, 'Elmar' apricot cultivar

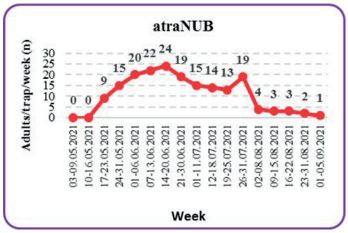

Figure 9. *Hedya nubiferana* flight dynamic, established with atraNUB pheromone traps at RSFG Constanta, 2021, 'Elmar' apricot cultivar

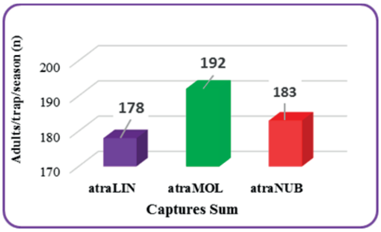

Fig. 10. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2021, 'Elmar' apricot cultivar

In the apricot experimental orchard, the attack by *Anarsia lineatella* was monitored and the flight dynamics and the total catches for the period May-September 2022 were realized. It is observed that the maximum of the flight curves, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees. The apricot of the flight *Anarsia lineatella* at 'Elmar' cultivar was registered in the second decade of June 2022, 44 butterflies, (Figure 11). In the apricot orchard, in the experiment area, the attack with *Cydia molesta* was monitored and the flight dynamics and the total catches were realized for the period May-August 2022. It is observed that the maximum of the flight curve, determined with the aid of the atraMOL pheromone trap for *Cydia molesta*, was recorded in the second decade of June, at 'Elmar' variety, 41 butterflies/trap/week (Figure 12). In the apricot orchard, the attack of the green bud moth (*Hedyia nubiferana*), a species with only one generation per year, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the second decade of June 2022, 27butterflies/trap/week (Figure 13). From the analysis of figure 14, it can be seen that the maximum value of the sum of the catches, 341 male butterflies/trap/season (2022), was recorded for the peach shoot moth *(Anarsia lineatella),* followed by the bud moth(*Cydia molesta*) with a sum of medium-high 255 male butterflies/trap/season, which indicates a reserve from the previous growing season. The same figure also shows the minimum level of the amount of peach moth (*Hedya nubiferana*) catches, namely 193 male butterflies/trap/season.

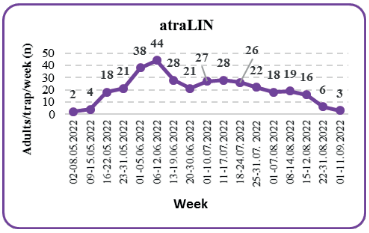

Figure 11. *Anarsia lineatelala* flight dynamic established with atraLIN at RSFG Constanta, 2022, 'Elmar' apricot cultivar

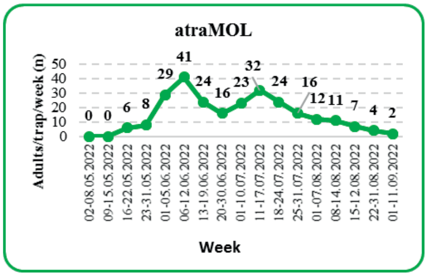

Figure 12. *Cydia molesta* flight dynamic, established with atraMOL pheromone trap at RSFG Constanta, 2022 'Elmar' apricot cultivar

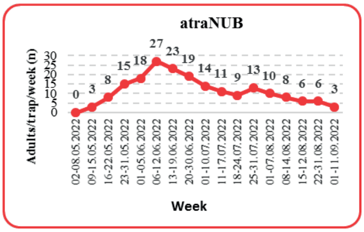

Figure 13. *Hedya nubiferana* flight dynamic established with atraNUB feromone traps at RSFG Constanta, 2022, 'Elmar' apricot cultivar

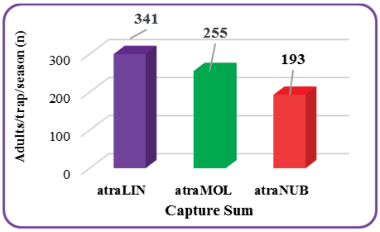

Figure 14. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2022, 'Elmar' apricot cultivar

In the apricot orchard from the experience, the attack with *Anarsia lineatella* was monitored and the flight dynamics and the total catches for the period May- September 2020 were realized. It is observed that the maximum of the flight curves, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees at 'Olimp' cultivar was registered in the first decade of July 2020, 39 butterflies/trap/week (Figure 15). In the experimental apricot orchard, the attack of *Cydia molesta* was monitored and the flight dynamics and the total catches were realized for the period May-September 2020. It is observed that the maximum of the flight curve, determined using the atraMOL pheromone trap for *Cydia molesta*, was recorded in the second decade of July on the 'Olimp' cultivar, 41 butterflies/trap/week (Figure 16). In the experimental apricot orchard, the attack of the green bud moth (*Hedyia nubiferana*), a species with only one generation per year, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the third decade of July 2020, 45 butterflies/trap/week (Figure 17). From the analysis of Figure 18, it can be seen that the maximum value of the amount of catches, 312 male butterflies/trap/season, was recorded for the bud moth (*Hedya nubiferana*), followed by the peach moth (*Cydia molesta*) with an average amount of catches of 308 male butterflies/trap/season. From the same figure, the minimum level of the sum of the captures of the peach shoot moth (*Anarsia lineatella*) can be noted, namely 301 male butterflies/trap/season.

The monitoring continued also in the year of 2021. In the apricot experimental orchard, the attack by *Anarsia lineatella* was monitored and the flight dynamics and the total catches for the period May-September 2021 were realized. It was observed that the maximum of the flight curve, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees for *Anarsia lineatella* at cultivar 'Olimp' was registered in the third decade of July 2021, 31 butterflies/trap/week (Figure 19). In the apricot orchard where the experiment was set up, the attack with *Cydia molesta* was monitored and the flight dynamics and the total catches were made for the period May-September 2021. It is observed that the maximum of the flight curve, determined with the aid of the atraMOL pheromone trap for *Cydia molesta*, was recorded in the third decade of July at the Olimp cultivar, 44 butterflies/trap/week (Figure 20). In the apricot orchard, the attack of the green bud moth (*Hedyia nubiferana*), a species with only one generation per year, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the fourth decade of July 2021, 51 butterflies/trap/week (Figure 21). From the analysis of Figure 22, it can be seen that the maximum value of the amount of catches, 303 male butterflies/trap/season, was recorded for the bud moth (*Hedya nubiferana*), followed by the peach moth (*Anarsia lineatella)* with an average amount of catches of 280 male butterflies/trap/season. From the same figure, the minimum level of the sum of the captures of the peach shoot moth (*Cydia molesta*) can be noted, namely 271 male butterflies/trap/season.

It is observed that the maximum of the flight curves, determined with the aid of the atraLIN pheromone trap installed in the crown of the trees. The apricot of the flight *Anarsia lineatella* at 'Olimp' cultivar was registered in the second decade of July 2022, 39 butterflies, (Figure 23). In the apricot orchard, in the experiment area, the attack with *Cydia molesta* was monitored and the flight dynamics and the total catches were realized for the period May-September 2022. It is observed that the maximum of the flight curve, determined with the aid of the atraMOL pheromone trap for *Cydia molesta*, was recorded in the first decade of July, at 'Olimp' cultivar, 47 butterflies/trap/week (Figure 24). In the apricot orchard, the attack of the green bud moth (*Hedyia nubiferana*), a species with only one generation per year, was monitored. The maximum flight curve, determined using the atraNUB pheromone trap, was recorded in the third decade of July 2022, 35 butterflies/trap/week (Figure 25). From the analysis of figure 26, it can be seen that the maximum value of the sum of the catches, 302 male butterflies/trap/season (2022), was recorded for the peach shoot moth (*Cydia molesta*), followed by the bud moth *(Anarsia lineatella),* with a sum of medium-high 292 male butterflies/trap/season, which indicates a reserve from the previous growing season. The same figure also shows the minimum level of the amount of peach moth (*Hedya nubiferana*) catches, namely 267 male butterflies/trap/season.

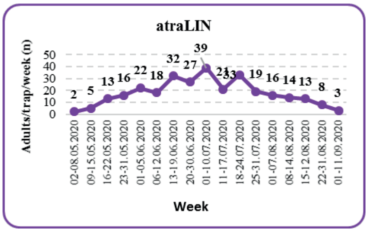

Figure 15. *Anarsia lineatelala* flight dynamic established with atraLIN at RSFG Constanta, 2020, 'Olimp' apricot cultivar

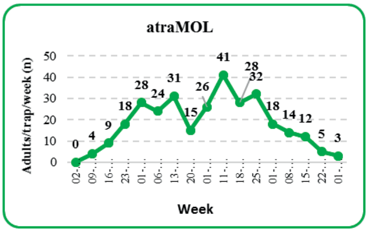

Figure 16. *Cydia molesta* flight dynamic, established with atraMOL pheromone trap at RSFG Constanta, 2020 'Olimp' apricot cultivar

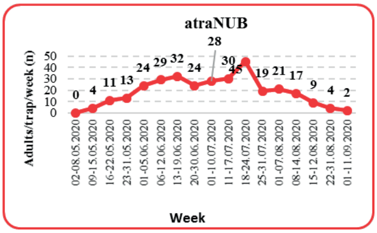

Figure 17. *Hedya nubiferana* flight dynamic established with atraNUB feromone traps at RSFG Constanta, 2020, 'Olimp' apricot cultivar

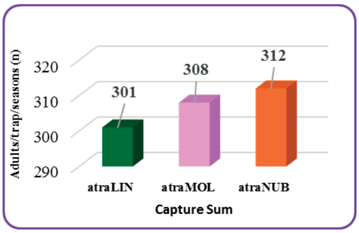

Figure 18. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2020, 'Olimp ' apricot cultivar

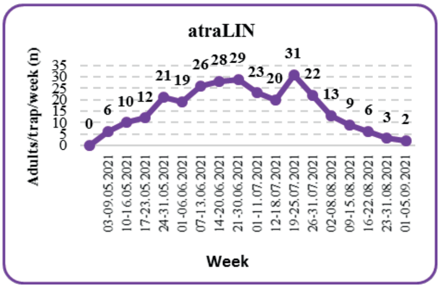

Figure 19. *Anarsia lineatelala* flight dynamic established with atraLIN pheromone traps at RSFG Constanta, 2021, 'Olimp' apricot cultivar

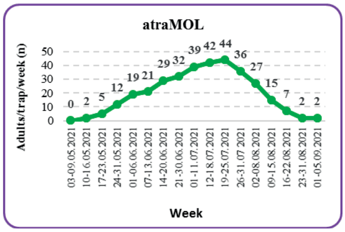

Figure 20. *Cydia molesta* flight dynamic, established with atraMOL pheromone traps at RSFG Constanta, 2021, 'Olimp' apricot cultivar

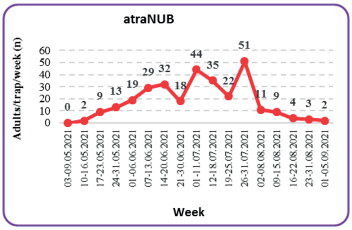

Figure 21. *Hedya nubiferana* flight dynamic, established with atraNUB pheromone traps at RSFG Constanta, 2021, 'Olimp' apricot cultivar

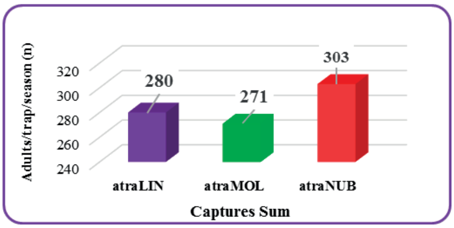

Figure 22. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2021, 'Olimp' apricot cultivar

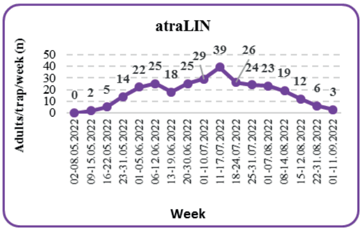

Figure 23. *Anarsia lineatelala* flight dynamic established with atraLIN pheromones trap at RSFG Constanta, 2022, 'Olimp' apricot cultivar

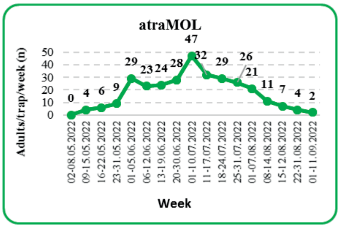

Figure 24. *Cydia molesta*, flight dynamic established with atraMOL pheromones trap at RSFG Constanta, 2022, 'Olimp' apricot cultivar

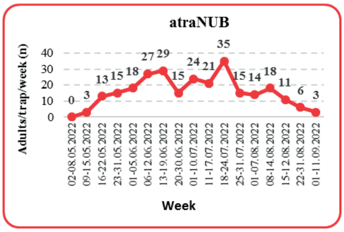

Figure 25. *Hedya nubiferana*, flight dynamic established with atraNUB feromones trap at RSFG Constanta, 2022 'Olimp' apricot cultivar

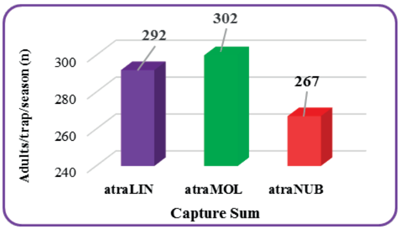

Figure 26. The amount of catches of harmful microlepidoptera at RSFG Constanta, 2022, 'Olimp' apricot cultivar

CONCLUSIONS

Monitoring harmful microlepidoptera in apricot orchards ispossible by using pheromonal traps specific to each species.

The synthetic sex pheromones atraLIN, atraMOL and atraNUB showed high potency of attraction, proving to be useful in knowing the level of peach moth populations (*Anarsia lineatella*), the peach moth *(Cydia molesta*), and the bud moth (*Hedya nubiferana*), the dynamics of populations and the application of chemical treatments at optimal times.

At 'Elmar' cultivar, the sum of catches per trap was higher in 2022, 341 male butterflies/trap/season was recorded for the apricot shoot moth (*Anarsia lineatella*, atraLIN), followed by the bud moth (*Cydia molesta*) with a sum of medium-high 254 male butterflies/trap/season and the minimum level of the amount of catches was recorded for the peach moth (*Hedya nubiferana*), namely 193 male butterflies/trap/season.

At 'Olimp' cultivar, the amount of catches per trap was higher in 2020, the maximum value of the amount of catches, 312 male butterflies/trap/season was recorded for the peach shoot moth (*Hedya nubiferana*), followed by the peach moth (*Cydia molesta*) with a medium-high capture amount of 308 male butterflies/trap/season, which indicates a reserve from the previous growing season and the minimum level of the capture amount was recorded for the bud moth (*Anarsia lineatella*), namely 301 male butterflies/ trap/season.

The results of this study provide an understanding of the key issues of environmental impact related to the maintenance of pathogens and pests below the economic threshold of damage, the reduction of toxic substances accumulated per ha, the environmental pollution, while also achieving balanced nutritional conditions for trees, in the species apricot, species with a good culture favorability in Dobrogea.

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increase profitability and ensure environmental protection).

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MORPHO-BIOCHEMICAL RESPONSE OF BLUEBERRY CULTIVAR HORTBLUE PETITE UNDER DROUGHT STRESS INDUCED BY PEG 6000

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Abstract

The in vitro response of the blueberry cultivar Hortblue Petite to drought stress induced by PEG 6000 was investigated in this study. PEG was added to the culture media before pH adjustment at the following concentrations: 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L. The culture medium used was Woody Plant Medium (WPM) with 100 mg/l Sequestren 138, 1 mg/L zeatin (Z), and a pH of 5. After 80 days of in vitro culture, the average number of shoots, shoot length, fresh weight (FW), dry weight (DW), water content (WC), chlorophyll (Chl a, Chl b), carotenoids (Caro), proline (Pro), total soluble sugars (TSS), total phenolic compounds (TPC), total flavonoids (TFC), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) were evaluated. Drought stress induced by PEG increased the number of proliferated shoots but harmed shoot length. A reduction in the content of photosynthetic pigments was also observed. Osmolytes, oxidative stress markers, and antioxidant compounds also indicated the effect of drought stress on the Hortblue Petite blueberry cultivar.

***Key words**: drought, hydrogen peroxide, malondialdehyde, proline, V. corymbosum.*

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum* L.) is the most commonly cultivated, commercially important, and biologically valuable species of the genus *Vaccinium*. In recent years, blueberries have become increasingly popular among consumers worldwide due to their flavor, high nutritional value, and health benefits (Ruzić et al., 2012; Mazurek et al., 2021; Ciucu-Paraschiv et al., 2023; Hera et al., 2023; Muñoz-Fariña et al., 2023; Shi et al., 2023). At the same time, highbush blueberry is one of the most sensitive species to water shortages in soils due to its superficial root system (Bryla & Strik, 2007; Sandoval et al., 2024). It is recognized that there are many varieties of *V. corymbosum*, but their response to drought depends largely on the genetic characteristics of each variety (Calderón-Orellana et al., 2023).

Drought is an important abiotic stress, caused by water imbalance in the ecosystem, which can have severe consequences in plants and lead to significant losses in the production of woody species (Vuksanović et al., 2022). Water stress affects the normal functionality of plants and induces morphological, physiological, and biochemical changes. A detailed understanding of the patterns and response mechanisms of plants to water stress is crucial for predicting the functionality and resilience of plants in the face of increasingly frequent drought episodes (Sun et al., 2020).

The in vitro screening condition is considered beneficial compared to field screening, although the drought effect may not be replicated at all stages of development through this method. Preliminary reports can predict the plant response to stress, and subsequent validation of responses to stress can be conducted under field conditions (Sahu et al., 2023).

Polyethylene glycol (PEG), a high molecular weight compound, is used to induce drought stress in different plants species under in vitro conditions such as: wild cherry (Vuksanović et al., 2022), fox grapes (Bilir et al., 2022), highbush blueberry (Molnar et al., 2022), cotton (Jaafar et al., 2021), vanilla (Martínez-Santos et al., 2021), sugarcane (Hernández Pérez et al., 2021), stevia (Ahmad et al., 2020), sweet violet (Darvishani et al., 2020), tomato (Naveed et al., 2019), olive (Silvestri et al., 2017).
Several biological parameters change while plants are subjected to drought stress and their study provides important data to understand the mechanisms by which plants can tolerate this phenomenon (Tafreshi et al., 2021). Along with the morphological changes of plants under drought stress, some biochemical parameters can define the resistance of plants to drought stress: photosynthetic pigments, antioxidant compounds, oxidative stress markers, or osmolytes (Coşkun, 2023; Eisa et al., 2023; Habuš Jerčić et al., 2023). In this context, the objective of this study was to evaluate the morphological and biochemical responses in in vitro blueberry cultivar Hortblue Petite shoots under drought stress conditions induced by PEG.

MATERIALS AND METHODS

In vitro culture and drought stress

Blueberry shoots (Hortblue Petite cv.) from the in vitro multiplication phase were used in this study. The shoots were propagated on Woody Plant Medium (WPM) (Lloyd & McCown, 1980) supplemented with 100 mg/L Sequestrene 138 and 1 mg/L zeatin (Z) according to the protocol described by Clapa et al., 2018. The experimental design included using different concentrations of PEG 6000 as follows: 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L. PEG 6000 was added to the culture media before adjusting the pH and then autoclaved. The culture medium was solidified with 0.5% (w/v) plant agar. The pH of the media was adjusted to 5 before adding agar.
The culture medium was distributed in glass culture jars (capacity 720 mL; diameter 9 cm and height 13.5 cm) which were closed with transparent polyethylene caps. Then, 15 explants (each 1.5-2 cm long) were inoculated into each culture jar. After inoculation, the culture vessels were incubated in the growth room with a controlled environment (21 ± 1°C, 32.4 $mmol \cdot m^{-2} \cdot s^{-1}$, 16-h photoperiod). All the chemicals and reagents were purchased from Duchefa BiochemieBV, The Netherlands.

Growth parameters

The *in vitro* growth response to drought stress was evaluated after 12 weeks by measuring the following parameters: shoot length (cm), average number of shoots/inoculum, fresh weight (FW) of shoots per explant (mg), dry weight (DW) and water content (WC). As a note, to obtain dry weight (DW), the plant material was dried for three days at 45°C and reweighed. The water content expressed as a percentage (WC%) was calculated using the formula published by Mazurek et al., 2021:
WC (%) = ((Fresh Weight - Dry Weight)/Fresh Weight) * 100

Biochemical analyses

Photosynthetic pigments

The content of Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Carotenoids (Caro) were determined via spectrophotometry, using fresh plant material from in vitro grown shoots (50 mg). The samples were weighed and homogenized and extracted with 90% acetone in water until the residue was colorless. Absorbance was read at 645, 663, and 450 nm using a Perkin Elmer Lambda 25 spectrophotometer. The following formulas were used to quantify the Chl a and Chl b:
Chl a mg/g FW = (11.75 × A663 – 2:35 × A645) × V/g
Chl b mg/g FW = (18.61 × A645 – 3.96 × A663) × V/g
where: A645 and A663 represent the optical density at a specific wavelength, V represents the volume of the extract (mL), and g represents sample weight (mg).
The concentration of total Caro was calculated according to the formula used by Britton et al., 1995:
X mg carotenoids = (A × V × 1000) / (A 1% 1 cm × 100),
where: A represents absorbance at 450 nm, V represents volume (mL), and A1% 1 cm = 2500 and expressed as mg Caro/g fresh material.

Antioxidant compounds

Preparation of methanolic extract: 0.25 g fresh plant material (*in vitro* grown shoots) was prepared in 2.5 ml methanol, vortexed for 30 sec, then sonicated for 15 min and centrifuged for 10 min at 10000 rot/min at room temperature. The obtained supernatant was filtered through a 0.45 μ nylon filter.

Total phenolic compounds (TPC). The content of TPC was determined by the Folin-Ciocalteu method (Dulf et al., 2015) using a Perkin Elmer Lambda 25 spectrophotometer. The reaction mixtures (200 µl sample, 250 µl Folin-Ciocalteu reagent, 700 µl Na_2CO_3 solution, and 2700 µl water) were incubated at room temperature for 45 min in the dark. After that, the absorbance was measured at 750 nm. Gallic acid (GA) was used as the standard. TPC contents were expressed as mg eq. GA g^{-1} FW.

Total flavonoid content (TFC). TFC was determined using the method developed by Zhishen et al., (1999). Briefly, 500 µl methanol extract was mixed with 200 µl $NaNO_2$ (5%); after 5 min, 200 µl $AlCl_3$ (10%) was added. After 5 min standing, 1500 µL NaOH (1M) and 1100 µl water were added and the absorbance was measured after 15 min, at 510 nm with methanol as blank and the TF contents were expressed as equivalents of the standard Quercetin (mg eq. Q g^{-1} FW)

Quantification of osmolytes.

Proline (Pro): 0.1 g fresh ground shoot material was homogenized in 3% (w/v) sulphosalicylic acid and centrifugated at 3000 rpm for 10 min; 2 mL of the supernatant was mixed with 2 mL acid ninhydrin, incubated in a water bath at 100°C for 1 h, chilled on ice, and then extracted with 4 mL toluene. The proline concentration was determined from a standard curve calculated in fresh weight according to Bates et al. (1973).

The absorbance was measured at 520 nm using toluene for blank, and Pro concentrations were expressed as µmol g^{-1} FW.

Oxidative stress markers

Malondialdehyde (MDA): 0.2 g of fresh plants were mixed with 3 mL of 0.1% trichloroacetic acid (TCA), mixed for 30 s followed by centrifugation at 10,000 rpm, 4°C for 10 min. An aliquot of 0.5 mL supernatant was transferred into another tube and mixed with 1.5 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The samples were incubated for 30 min at 95°C in a constant temperature water bath and then cooled in an ice bath for 5 min. After centrifugation at 10,000 rpm for 10 min at 4°C, the absorbance of the obtained supernatant was detected at 532, 600, and 450 nm using a Lambda 25 (Perkin-Elmer Singapore) UV-Vis spectrophotometer. MDA concentrations were determined using the equation: $MDA = 6.45(A_{532}-A_{600})-0.56A_{450}$

MDA concentrations were calculated in nmol g^{-1} FW (Hodges et al., 1999).

Hydrogen peroxide (H_2O_2). For H_2O_2 quantification 0.1 g of fresh shoots were homogenized in an ice bath with 5mL of 0.1% (w/v) TCA and the extract was centrifuged at 12000 g for 15 min. 0.5 mL of the supernatant was combined with 0.5 mL of 10 mM potassium phosphate buffer (pH = 7) and 1 mL of 1M potassium iodide (KI) from Duchefa Biochemie BV (Haarlem, The Netherlands). After incubation for 30 min at room temperature, the absorbance was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve using different concentrations of H_2O_2.

Antioxidant activity

DPPH free-radical-scavenging activity was performed using the method described by Brand-Williams et al. (1995) with slight modifications. First, 35 µL of each leaf methanolic extract was mixed with 250 µL of freshly prepared DPPH solution (80 µM in methanol). The absorbance was measured after 30 min of standing, under dark conditions, at 515 nm, using a Synergy HT microplate reader (BioTeK®, USA) and methanol as blank. In the DPPH assay, the antioxidant activity of the extracts was evaluated using the calibration curve performed with Trolox, and then the absorbance was recorded for all the tested extracts, to calculate the percentage inhibition (expressed as percentage inhibition of the DPPH radicals). The percentage inhibition (I%) was calculated as $I\% = [(AB-AA)/AB]\times 100$, where: AB = absorbance of blank and AA = absorbance of methanolic extract.

Statistical analysis

One-way analysis of variance (ANOVA) was performed. Post hoc testing for the ANOVAs was performed using Tukey's honestly significant difference test (Tukey's test) using a $P<0.05$ significance level to determine the statistically significant differences between the means. Values shown (in text and figures) are means ± SE (standard error).

RESULTS AND DISCUSSIONS

In vitro growth parameters

The growth parameters were measured after ten weeks of *in vitro* culture on WPM medium supplemented with 100 mg/L Sequestrene and 0.5 mg/L Z in various PEG 6000 treatments. These treatments included 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, and 50 g/L PEG 6000 (Figure 1). The measured parameters included the number of proliferated shoots per initial inoculum, shoot length, fresh weight, and dry weight per proliferated inoculum, as well as the water content (Table 1). As observed in Table 1, hydric stress resulted in an increase in the number of shoots per inoculum in all applied PEG treatments. The highest number of proliferated shoots was obtained when the culture medium was treated with 20 g/L PEG (17.09±0.52) and decreased with the increasing concentration of PEG, reaching 13.21±0.76 under the treatment with 50 g/L PEG. Contrary to the number of proliferated shoots, their length decreased with the increasing concentration of PEG, such that in the treatment with 50 g/L PEG, the length of the shoots was halved (Table 1).

In all PEG treatments, there was an increase in fresh weight compared to the control without PEG. The largest increase was observed in the treatment with 40 g/l PEG (170.43±5.40 mg), which was 53% higher than in the culture medium without PEG (Table 2).

In terms of shoot water content, it increased with the rising PEG concentration. Shoots grown without PEG showed the lowest water percentage (75.44%), while it increased by 11.7% in shoots grown in a medium treated with 50 g/l PEG (Table 2).

Therefore, among the morphological parameters, shoot length was strongly affected by drought stress at all PEG concentrations (Table 1). Growth inhibition is a common response of plants subjected to water stress and has been reported for various plant species cultivated in vitro on culture media under different PEG concentrations. Thus, similar to blueberry, water stress reduced shoot length in *Vanilla planifolia* (Martínez-Santos et al., 2021), *Saccharum* spp. Hybrids (Hernández-Pérez et al., 2021), *Viola odorata* (Darvishani et al., 2020), and *Prunus avium* (Vuksanović et al., 2022). Water stress induced by PEG also had a negative impact on shoot length of *Myrtus communis* (Tafreshi et al.,2021).

In present study, a significant increase in the number of proliferated shoots/initial inoculum as well as in fresh weight was observed under all concentrations of PEG. Few previous reports have focused on the increase in the number of proliferated shoots under drought stress conditions. This may be due to the fact that PEG-supplemented culture media did not contain growth regulators. Contrary to our findings, Vuksanović et al. (2022) reported a lower number of proliferated shoots under drought stress induced by PEG (20 and 50 g/l) compared to the culture medium without PEG (MS supplemented with 1 mg/l BA and 0.5 mg/l kinetin) for two clones of Wild cherry. Also, Tafreshi et al. (2021) show that the total number of proliferated shoots of *M. communis* was negatively affected by the content of PEG in the culture medium. Shoot number was reduced for explants grown in media containing 3% PEG and 6% PEG compared to control explants without PEG.

However, *Stevia rebaudiana* behaved similarly under drought stress conditions induced by PEG and had the highest average number of nodes, leaf number, and the highest fresh weight of shoots under the 4% PEG treatment followed by the results obtained under 2%, 1%, and 0.5% PEG 6000 treatments (Ahmad et al., 2020).

Biochemical analysis

Significant differences were observed in the contents of Chl a, Chl b, and Caro in *V. corymbosum* shoots exposed to different concentrations of PEG 6000. The highest contents of Chl a (1.48±0.06 mg/g FW), Chl b (0.64±0.03 mg/g FW), and total Caro (0.88±0.062 mg/g FW) were recorded in shoots grown in the culture medium without PEG. The lowest contents of photosynthetic pigments were recorded on culture media with 50 g/l PEG, with the general tendency being a decrease in the concentration of photosynthetic pigments with increasing PEG concentration (Table 2). The content of photosynthetic pigments is an important indicator of plant physiological status and diminished levels of these pigments have often been linked to drought conditions (Vuksanović et al., 2022). The decrease in Chl a and Chl b content in blueberry shoots, due to water stress, induced by the five concentrations of PEG in our study aligns with findings from various other studies conducted on different species. For example, Martínez-Santos et al. (2021) observed reduced levels of Chl a, Chl b, and total chlorophyll in *Vanilla planifolia* under drought stress induced by 1%, 2%, and 3% PEG. Similarly, Gao et al. (2020) documented the inhibitory effect of drought on the concentrations of Chl a and Chl b in *Dendrobium officinale* across different drought stress conditions (10, 30, and 50 g/l PEG). This decrease in photosynthetic activity under stress conditions can be attributed to stomatal closure, which is also a response mechanism to avoid energy waste (Martínez-Santos et al., 2021).

Figure 1. Effect of different concentrations of polyethylene glycol (PEG 6000) on the growth of the *in vitro* culture of highbush blueberry, cv Hortblue Petite: (a) 0 g/L PEG 6000; (b) 10 g/L PEG 6000; (c) 20 g/L PEG 6000; (d) 30 g/L PEG 6000; (e) 40 g/L PEG 6000; (f) 50 g/L PEG 6000

Table 1. Growth parameters measured after ten weeks of *in vitro* drought stress treatments in the Hortblue Petit highbush blueberry variety. Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$)

Treatments	Parameters									
	No. of shoots/ inoculum	% of reduction*	Length of shoots (cm)	% of reduction	Fresh weights (mg)	% of reduction	Dry weights (mg)	% of reduction	Water content %	% of reduction
0 PEG	8.80±0.56 a	0	1.99±0.56 c	0	110.23±3.25 a	0	25.03±4.32 ab	0	75.44±3.39 a	0
10 g/l PEG	16.27±1.00cd	-84.85	1.49±1.00 b	25.10	141.93±6.49 b	-28.76	27.20±1.87 c	-8.66	79.88±1.32 b	-5.89
20 g/l PEG	17.09±0.52 d	-94.19	1.08±0.52 a	45.62	147.10±6.82 b	-33.44	26.17±1.98 bc	-4.53	81.64±0.82 c	-8.21
30 g/l PEG	15.69±0.42 c	-78.28	0.96±0.42 a	51.79	118.13±4.47 a	-7.17	23.43±5.64 a	6.39	80.87±2.13 bc	-7.20
40 g/l PEG	16.78±1.11 d	-90.66	0.98±1.11 a	50.86	170.43±5.40 b	-54.61	28.87±7.83 d	-15.26	81.28±1.06 c	-7.74
50 g/l PEG	13.21±0.76 b	-50.09	0.99±0.76 a	50.00	149.87±3.23 b	-35.95	24.20±5.92 a	3.33	84.04±1.00 d	-11.40

*% - Percentages of reduction - the control average (0 PEG) was considered 100%)

Table 2. Effect of *in vitro* drought stress in the Hortblue Petit highbush blueberry shoots on contents of photosynthetic pigments (chlorophyll a - Chl A, chlorophyll b - Chl b, carotenoids - Caro), proline (Pro), malondialdehyde (MDA), hydrogen peroxide (H_2O_2)

Treatments	Biochemical analysis					
	Chl A (mg/g FW)	Chl B (mg/g FW)	Caro (mg/g FW)	Pro (µmole proline/g FW)	MDA (nmol/g FW)	H_2O_2 (µmol/g FW)
0 PEG	1.48±0.06 e*	0.64±0.032 d	0.88±0.062 d	4.59±0.109 a	339.45±0.001 a	236.0±0.003 a
10 g/l PEG	0.63±0.06 b	0.45±0.010 c	0.41±0.020 ab	4.95±0.034 b	473.21±0.012 b	373.3±0.044 b
20 g/l PEG	0.86±0.01 d	0.32±0.011 b	0.53±0.011 c	5.23±0.123 c	502.88±0.001 bc	481.9±0.075 c
30 g/l PEG	0.80±0.01 c	0.31±0.012 b	0.50±0.042 bc	6.50±0.041 d	519.17±0.001 bc	514.5±0.031 d
40 g/l PEG	0.63±0.03 b	0.24±0.033 a	0.41±0.033 ab	6.92±0.034 e	558.10±0.001 c	577.5±0.021 e
50 g/l PEG	0.55±0.06 a	0.21±0.028 a	0.36±0.058 a	7.69±0.507 f	835.75±0.001d	599.7±0.007 e

*Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$

As shown in Table 2, Pro accumulation was significantly higher in plants stressed by drought compared to those grown in the culture medium without PEG. The highest amount of proline (7.69 µmole proline/g FW) accumulated in the shoots grown on the culture medium with 50 g/l PEG, which was 1.68 times higher than the control.

Furthermore, a positive correlation can be observed between Pro accumulation and increasing drought levels. The highest Pro accumulation was recorded at maximum drought levels, similar to observations in other species subjected to drought stress induced by PEG (Piwowarczyk et al., 2014; Razavizadeh et al, 2019; Jiroutova et al., 2021). However, the proline content of *Myrtus communis* shoot significantly increased 5.7-fold under 3% PEG treatment compared to the control without PEG. In contrast, in the 6% PEG treatment, proline content was considerably lower compared to the 3% PEG treatment and was not statistically different from the control (Tafreshi et al., 2021). These results suggest that Pro has a role in plant drought stress tolerance mechanisms and the accumulation of Pro in plants is a physiological response to drought stress manifested by reduced plant growth.

Oxidative stress markers, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) exhibited a significant increase in shoots grown on culture media treated with all concentrations of PEG compared to culture media without PEG (Table 2). MDA reached its highest value on the culture medium with 50 g/l PEG (835.75±0.001 nmol/g FW), which was twice as high as the control without PEG (339.45±0.001 nmol/g FW). A similar response to drought stress was observed for H_2O_2, with concentrations of 599.7±0.007 µmol/g FW in shoots grown on culture medium supplemented with 50 g/l PEG, compared to 236.0±0.003 µmol/g FW in shoots of blueberries grown on culture medium without PEG. The results of this study are consistent with those reported in previous studies. For example, MDA content in *Vigna mungo* plants increased to the maximum level with increasing severe concentrations of PEG where a 20% increase was observed compared to the control (Jothimani & Arulbalachandran, 2020). Similarly, osmotic stress significantly increased MDA content relative to the control in the cucurbit species, and the highest MDA content was observed under the highest concentration of PEG (Tajaragh et al., 2022). Additionally, the highest content of H_2O_2 was obtained under the highest concentration of PEG added to the culture medium for the three Iranian *Cucurbita* sp. (Tajaragh et al., 2022), which is in accordance with our results.

In the case of total flavonoids (TF) and total phenolic content (TPC), Table 3 demonstrates that the highest levels (TPC 9.07±0.044 mg eq. GA/g FW and TF 4.67±0.022 mg Q/g FW) were observed in blueberry shoots grown in culture medium supplemented with 50 g/l PEG 6000. Conversely, the lowest levels of antioxidant activities (TPC 8.26±0.101 mg eq. GA/g FW and TF 3.69±0.005 mg Q/g FW) were recorded in blueberry shoots grown in the medium containing 10 g/l PEG 6000. In general, TPC and TFC content and DPPH in the blueberry shoots were reduced at mild stress and increased at intense osmotic stress conditions. The highest level in both traits was obtained in the shoots treated with 50 g/l PEG (Table 3).

The accumulation of secondary metabolites, such as total flavonoids (TF) and total phenolic content (TPC), is crucial for plant adaptation to environmental stressors and serves a vital role in protection against free radicals. Previous studies have shown that different plant species exhibit various responses in terms of secondary metabolite accumulation under stress conditions.

Table 3.Total phenolic content (TPC), total flavonoid content (TF), and DPPH activity of the Hortblue Petit highbush blueberry shoots under *in vitro* drought stress

Treatments	TF (mg Q/g FW)	TPC (mg eq. GA/g FW)	DPPH (I%)
0 PEG	4.56±0.083 b	8.93±0.098 b	92.42
10 g/l PEG	3.69±0.005 a	8.26±0.101 a	86.27
20 g/l PEG	3.70±0.192 a	8.37±0.552 a	88.37
30 g/l PEG	4.52±0.028 b	8.66±0.443 a	77.12
40 g/l PEG	4.54±0.114 b	8.98±0.016 b	75.42
50 g/l PEG	4.67±0.022 b	9.07±0.044 c	87.45

*Values are presented as means ± SE. Different lowercase letters indicate significant differences between treatments for each determined variable according to the Tukey test ($p < 0.05$).

For instance, Vuksanović et al. (2022) observed lower levels of TF and TPC in *Prunus avium* grown in vitro under stress conditions compared to the control without PEG. Similarly, Puente-Garza et al. (2017) reported a decrease in TF content under drought stress in *Agave salmiana*. As in the case of blueberry, *Stevia rebaudiana* had the highest content in TPC, TFC, TAC, TRP, and DPPH-FRSA, (TPC 15.03 µg/mg, TFC 13.01 µg/mg, TAC 22.26 µg /mg, TRP 19.87 pg/mg and DPPH-FRSA 95.27%) in shoots grown in MS medium supplemented with 4% PEG 6000 stress. It was followed by the amounts obtained under 2% PEG 6000 stress and later, 1% PEG 6000 stress, followed by 0.5% PEG 6000 stress. The lowest amount of antioxidant activities (TPC 6.97 µg/mg, TFC 4.44 µg/mg, TAC 13.7 µg/mg, TRP 10.22 µg/mg, and DPPH-FRSA 48.03%) was elucidated by shoots grown in PEG60-deficient medium (Ahmad et al., 2020).

CONCLUSIONS

In vitro drought stress induced by PEG 6000 had effects on growth and biochemical determinations evaluated in *V. corymbosum*. The most significant parameters that responded to drought stress in our study were: shoot length, shoot number, content of photosynthetic pigments, proline, malondialdehyde, and hydrogen peroxide. A reduction in proliferated shoot length and an increase in shoot number were observed on the WPM + 1 mg/l Z culture medium supplemented with 10, 20, 30, 40, and 50 g/l PEG. The content of photosynthetic pigments decreased, while the content of proline, malondialdehyde, and hydrogen peroxide increased with the increasing concentration of PEG 6000 in the culture medium. The results obtained suggest that the in vitro screening technique of highbush blueberries for drought tolerance, utilizing PEG as a stress agent, could serve as an alternative method for the early selection of drought-resistant cultivars. However, for validation, field research is recommended in line with practices for other species.

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COMPARATIVE STUDY IN THE NURSERY OF VEGETATIVE PLUM ROOTSTOCKS, ‘MIRODAD 1’ AND ‘SAINT JULIEN A’

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Abstract

In Romania, for plum species, the last registered vegetative rootstock was ‘Mirodad 1’. The purpose of the research was to study the behavior of the ‘Mirodad 1’ vegetative rootstock compared to the ‘Saint Julien A’ rootstock at propagating by softwood cuttings, at planting and grafting in nursery conditions from the Southern part of Romania. In the nursery, the interaction of the two rootstocks with the plum varieties ‘Tuleu gras’, ‘Jojo’ and ‘Romanța’ was evaluated. The indicators monitored were the rooting percentage of softwood cuttings, viable rootstocks remaining after planting and growth indicators of grafted trees (height, cross-section area of the trunk, number and length of shoots). The results of the propagation showed that the ‘Mirodad 1’ rootstock has a significantly higher rooting percentage than the ‘Saint Julien A’ rootstock. After grafting, the ‘Mirodad 1’ rootstock induces the same vigor as the ‘Saint Julien A’ rootstock to the ‘Tuleu gras’ and ‘Romanța’ cultivars. In the case of the ‘Jojo’ cv., the ‘Mirodad 1’ rootstock induces greater vigor than the ‘Saint Julien A’ rootstock.

Key words: *grafted trees, plum, propagation, vegetative rootstocks, vigor.*

INTRODUCTION

At international level, there is a permanent concern to obtain vegetative rootstocks which have easy propagation, induce low vigor to the grafted cultivars and have resistance to diseases and pests specific and also to abiotic factors. Among them, were noted the following rootstocks: ‘Krymsk 1’ (‘VVA-1’), ‘Krymsk 86’, ‘Krymsk 2’ (‘VSV-1’) in Russia; ‘Mirobolan 29 C’, ‘Citation’, ‘AP-1’, ‘Marianna’ group in the USA; ‘Myrobolan A’, ‘Micronette’, ‘Dospina 235’, ‘Docera 6’ in Germany; ‘Myrocal’, ‘INRA GF31’, ‘INRA Saint Julien 655/2’, ‘Myram’, ‘Ishtara’, ‘Prumina’, ‘Ferlenain’ in France; ‘Myrobolan B’, ‘Saint Julien A’, ‘Pixy’, ‘Brompton’, in Italy; ‘MRS2 /5’, ‘Penta’, ‘Tetra’ in England; ‘MI-BO-1’, ‘MY-KL-A’ in Slovakia; ‘Besztercei szilva’ in Hungary; ‘Wavit’ in Austria (Hartmann et al., 1997; Botu et al., 2004; Czinege et al., 2012; Necas and Krska, 2013; Zamfirescu, 2022).

Although at international level there are numerous plum vegetative rootstocks, due to the pedo-climatic conditions from Romania there is a permanent concern for the creation of plum rootstocks with vegetative propagation. From Romanian rootstock breeding program resulted numerous and valuable generative (‘Roșior văratic’, ‘Buburuz’, ‘Renclod verde’, ‘Voinești B’, ‘Scolduș’, ‘Porumbar de Iași’, ‘Mirobolan dwarf’, ‘Mirobolan C5’, ‘BN4Kr’, ‘Oteșani 8’) and vegetative rootstocks (‘Oteșani 11’, ‘Miroval’, ‘Rival’, ‘Corval’, ‘Oltval’, ‘Pinval’, ‘Corcoduș 163’, ‘Mirodad 1’) (Mazilu et al., 2013, 2014). In the current period, the research trend is to obtain vegetative rootstocks, which are identical to the mother plant and induce to the grafted trees low vigor and uniformity.

The purpose of the research was to study the behavior of the ‘Mirodad 1’ vegetative rootstock compared to the ‘Saint Julien A’ rootstock at propagating by softwood cuttings, at planting and grafting in nursery conditions from the South part of Romania.

‘Mirodad 1’, approved as a vegetative rootstock, was obtained by controlled hybridization between ‘Mirobolan dwarf’ (*Prunus cerasifera*) and ‘Adaptabil’ (*Prunus besseyi*).

MATERIALS AND METHODS

Field trial and plant material

The observations were carried out at the Research Institute for Fruit Growing Pitești, in

the experimental fields of the nursery, located in the Southern part of Romania, at 44°51'30" North latitude and 24°52' East longitude.
In the nursery, the interaction of the two rootstocks (‘Mirodad 1’ and ‘Saint Julien A’) with the three plum cultivars (‘Tuleu gras’, ‘Jojo’ and ‘Romanța’) was evaluated.

Measurements

Between 2019-2021, the following parameters were appreciated and measured: the rooting percentage of softwood cuttings; the cuttings remaining after planting in the first field of the nursery and the influence of the rootstocks on the growth of the three cultivars in second field of the nursery.
The softwood cutting was carried out at the beginning of July. The cuttings, 25 cm long, were planted on high beds, in the sand substrate, under artificial fog conditions. Two experimental variants have been used: V0 – control without biostimulatory; V1 – treated with Radistim V2 (ANA based rooting biostimulatory) applied in powder form. For each variant, 100 cuttings were planted at a distance of 8 x 5 cm. The rooting percentage was determined in November, at harvest time, when the root of the cutting is lignified. In the springs of 2020 and 2021, the cuttings were planted at a distance of 90 x 15 cm in the first field of nursery. The rootstocks remaining after planting were determined at the beginning of June. In August, 2020, the three plum cultivars (‘Tuleu gras’, ‘Jojo’ and ‘Romanța’) were grafted using the chip-budding method (60 rootstocks for each rootstock - cultivar combination). The indicators monitored before harvesting of the grafted trees were the vigor and the shooting capacity for each cultivar - rootstock combination. The vigor was expressed by the average trees height and the trunk cross-sectional area (TCSA) at 20 cm above the grafting point. The shooting capacity was appreciated by the number and length of shoots/tree (over 50 cm above the grafting point).

Statistical analysis

The data were included in an Excel database. The significance of the influence of the experimental factors on cutting, planting and grafting was highlighted using the ANOVA variance analysis test. Differences between means were highlighted at 0.05% level of probability.

RESULTS AND DISCUSSIONS

Softwood cuttings propagation

‘Saint Julien A’ is a vegetative rootstock widely used throughout the world due to its good compatibility with plum varieties, the low vigor induced to the cultivars and the fact that it does not issue layers in the orchard. It is propagated vegetative both by macropropagation with hardwood or softwood cuttings (Markovski et al., 2015) and by micropropagation (*in vitro*) (Nacheva et al., 2023).
‘Mirodad 1’ is a vegetative plum rootstock with low vigor, resistant to diseases and well adapted to the soils of Romania. It is compatible with most European plum cultivars, and does not emit suckers in the orchard (Duțu et al., 2016).
Figure 1 shows images of cuttings rooted on the two rootstocks studied.

a **b**

Figure 1. ‘Saint Julien A’ (a) and ‘Mirodad 1’ (b) – rooted cuttings

The ‘Saint Julien A’ rootstock propagation by softwood cuttings had a rooting percentage between 2.15% in the V0 variant and 55.56% in the V1 variant. The ‘Mirodad 1’ rootstock had a significantly higher rooting percentage than ‘Saint Julien A’, in the both variants, ranging between 72.38% (V0) and 98.99% (V1) (Figure 2).

Figure 2. The influence of the rootstock on the rooting percentage
(Notes: Means with different letters between rootstocks are statistically different, P≤0.05)

The best rooting percentage was recorded in 2020 in the V1 variant treated with Radistim V2 with a maximum of 98.99% at ‘Mirodad 1’ and 55.56% at ‘Saint Julien A’ (Figure 3). Our results are similar with results obtained by Lopez-Bucuio et al., 2003, regarding use of biostimulators.

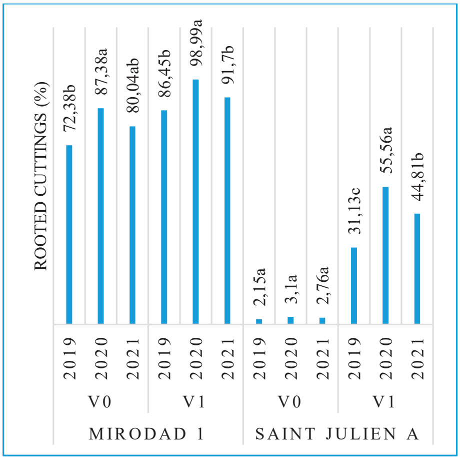

Figure 3. The variation of the rooting percentage of the softwood cuttings in the experimental period
(Notes: Means with different letters between years are statistically different, P≤0.05)

The success of the rooting process depends on numerous factors such as the health and nutrition of the mother plants, the vegetation stage of the shoot in which the softwood cuttings was made, the substrate and rooting biostimulator used, the amount of water and the watering interval in the greenhouse, etc.
Variation in either of these factors can result in changes in rooting percentage from year to year.
In the case of this experience, regardless of the substrate, the cuttings' moment and the biostimulator used were the same between the cutting years, significant differences appear both in the ‘Mirodad 1’ rootstock in both variants and in ‘Saint Julien A’ in the V1 variant. In the present case, the reason could be the amount of water and watering intervals during the rooting period.
The statistical data showed that in the V1 variant – treated with Radistim V2 the rooting percentage is significantly higher than in the V0 variant – without biostimulator (Figure 4).

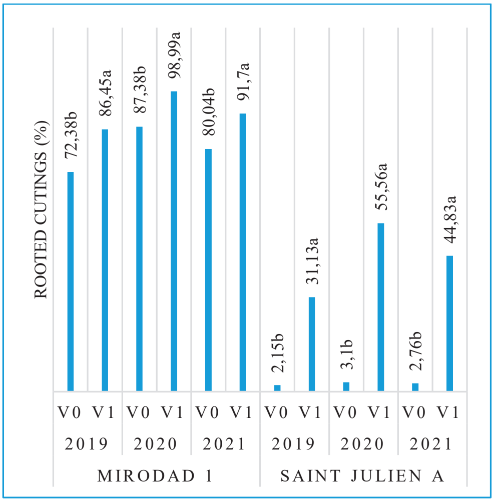

Figure 4. The influence of the biostimulator on the rooting capacity of rootstock cutting
(Notes:Means with different letters between variants are statistically different, P≤0.05)

Planting of rooted cuttings

In the springs of 2020 and 2021, the rootstocks were planted in the first field of the nursery at a distance of 90 cm between rows and 15 cm between plants per row, in order to graft in August. The evaluation of the rootstocks remaining after planting showed that in the case of ‘Saint Julien A’ rootstock there was a significantly higher percentage (97.77%) than in the case of ‘Mirodad 1’ rootstock (95.5%), average values over two years (Figure 5).

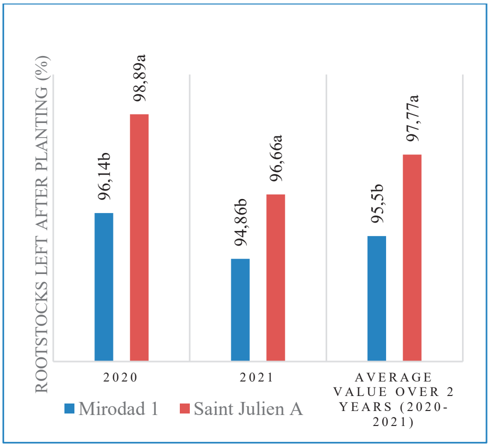

Figure 5. Rootstocks left after planting for grafting
(Notes: Means with different letters between rootstocks are statistically different, P≤0.05)

The vigor induced by the rootstock to the grafted cultivars

In August, three plum cultivars ‘Tuleu gras’, ‘Romanța’ and ‘Jojo’ were grafted, using the chip-budding method, on ‘Mirodad 1’ and ‘Saint Julien A’ rootstocks. ‘Tuleu gras’ and ‘Romanța’ are Romanian cvs. spread in commercial orchards from Romania. ‘Jojo’ cv., registered in Germany, was recently introduced in our country due to Plum Pox Virus resistance (Butac et al., 2018).

In order to evaluate the influence of the studied rootstocks on the three plum cultivars, tree vigor and shooting capacity, in the second field of the nursery, were carried out (Table 1).

The average height of the trees varied between 164.71 cm for the ‘Romanța’ cv. grafted on ‘Saint Julien A’ and 102.73 cm for the ‘Jojo’ cv. grafted on the same rootstock.

The average surface of the trunk section varied between 159.54 mm^2 la ‘Romanța / Mirodad 1’ combination and 59.51 mm^2 la ‘Jojo / Saint Julien A’ combination. It can be seen from Table 1 that regarding the trees vigor, expressed by the trees height and the surface of the trunk section, the cultivars grafted on the ‘Saint Julien A’ rootstock have a slightly smaller vigor than those grafted on ‘Mirodad 1’, the differences being insignificant. The results regarding the influence of the ‘Saint Julien A’ rootstock on the vigour of ‘Jojo’ cv. are similar with other reported by Blazek et. al., 2009. Regarding ‘Romanța / Mirodad 1’ combination similar results were reported by Butac et al. in 2023.

The shooting capacity was the highest at ‘Tuleu gras’ cv. grafted on ‘Mirodad 1’ (4.7 shoots/tree). At the other extreme being ‘Jojo’ cv. grafted on ‘Saint Julien A’, the shooting capacity being very low, almost non-existent (0.09 shoots/ tree).

The average length of the shoots on the tree varied between 31.56 cm for the ‘Tuleu gras’ cv. grafted on ‘Saint Julien A’ and 1.36 cm for the ‘Jojo’ cv. grafted on the same rootstock.

Following the statistical analyses, it was observed that there were no significant differences between the trees of ‘Tuleu gras’ and ‘Romanța’ cvs. grafted on ‘Mirodad 1’ and ‘Saint Julien A’ rootstocks in case of tree vigor and shooting capacity.

In the case of the ‘Jojo’ cv., it is observed that the trees grafted on ‘Mirodad 1’ have significantly higher vigor and shooting capacity than the trees grafted on ‘Saint Julien A’.

Although there are differences between the Romanian cultivars and the ‘Jojo’ cv., still the average effect of the rootstock on the three cultivars showed that ‘Mirodad 1’ rootstock induces the same vigor and shooting capacity in the nursery as ‘Saint Julien A’ rootstock (Table 1).

Table 1. The influence of the rootstock on the growth indicators of the grafted tree

Growth indicators	Rootstock	Cultivar			Average rootstock effect
		Tuleu gras	Romanța	Jojo	
Tree height (cm)	Mirodad 1	136.81a	152.94a	130.62a	**140.12a**
	Saint Julien A	143.46a	164.71a	102.73b	**136.96a**
TCSA (mm^2)	Mirodad 1	124.17a	159.54a	158.02a	**147.24a**
	Saint Julien A	125.15a	159.15a	59.51b	**114.60a**
Number of shoots/ tree	Mirodad 1	4.78a	2.5a	2.68a	**3.32a**
	Saint Julien A	3.31a	2.88a	0.09b	**2.09a**
Length Of shoot /tree (cm)	Mirodad 1	18.13a	29.57a	20.68a	**22.79a**
	Saint Julien A	31.56a	26.49a	1.36b	**19.80a**

Notes: The values are compared vertically. Means with a different letter are statistically different (P≤0.05).

Figure 6 represent tree of ‘Romanța’ grafted on ’Mirodad 1’ at harvest moment.

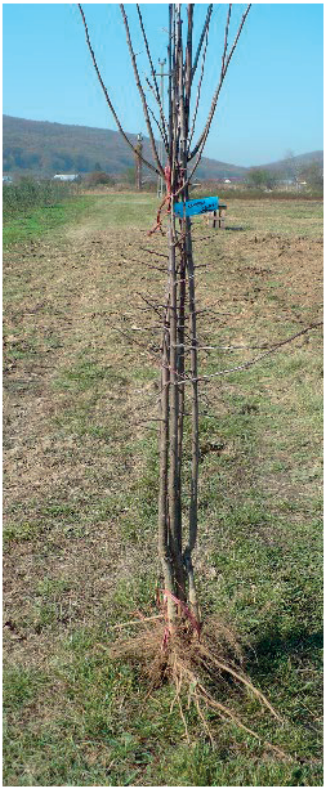

Figure 6. Romanța/Mirodad 1- grafted trees

CONCLUSIONS

The 'Mirodad 1' rootstock has a significantly higher rooting percentage than 'Saint Julien A', the highest value being in the variant with the rooting biostimulator based on naphthyl acetic acid (Radistim V2).

The percentage of rootstocks remaining for grafting after planting was greater than 95%, being significantly better for the 'Saint Julien A' rootstock than for the 'Mirodad 1' rootstock. The two rootstocks induce in the nursery vigor similar to the trees of the 'Tuleu gras' and 'Romanța' cvs. instead of the trees of 'Jojo' cv. grafted on 'Saint Julien A' have lower vigor and shooting capacity than trees of the same cultivars grafted on 'Mirodad 1' rootstock.

In conclusion, as a medium effect, rootstock 'Mirodad 1' induces, in the nursery, grafted cultivars similar vigor and shoot capacity to rootstock 'Saint Julien A', but with easier propagation by softwood cuttings.

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SENSORIAL ANALYSIS FOR SOME ROMANIAN AND FOREIGN BLUEBERRY VARIETIES

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Abstract

Blueberries are considered super-food because of their richness in antioxidants and vitamin C, essential for strengthening the immune system and protecting the brain. Breeders strive to improve the blueberries' fruit quality treats, so these fruits will have flavors and unique tastes, considerable size, and intense colors, which makes them even more appreciated by consumers. The firmness of fruits, their juiciness, taste, aroma, size, and color are all indicators used to appreciate the 30 blueberry varieties used in this study. Eight Romanian varieties and 22 foreign varieties were analyzed in a testing session in August 2023 with 51 accessors. The highest ranking is attributed to the 'Brigitta' variety. The 'Pink Lemonade' variety scored the best results for flavor, taste, firmness, and juiciness. The Romanian variety 'Delicia' was in the top 3 for 3 indicators. The results of the present study could be used by breeders to select promising varieties as genitors in blueberry breeding programs.

Key words: *Vaccinium corymbosum L., organoleptic evaluation, consumer preferences.*

INTRODUCTION

Blueberries are extremely appreciated for their special taste and their benefits for human health. It is well known that these small fruits can reduce the risks of diabetes (Nunes et al., 2021), cancer (Davidson et al., 2018), and cardiovascular diseases (Del Bo' et al., 2022) and because of these effects, they become very popular. This popularity led to the high bush blueberry plantations increased cultivated surface, including the protected area cultivation, with the marked advantage of delaying the last harvest (Asănică et al., 2017) and making the fruits available all year round.

The taste of blueberries is related to their chemical composition, the sugars, and organic acid combinations, whereas the physicochemical characteristics effects on flavor are highly influenced by the environmental conditions (Marina & Niculina, 2020), (Zhang et al., 2020), (Hera et al., 2022) (Shi et al., 2023). Some fruit production can be delayed by climatic characteristics and some years do influence the development of blueberry vegetation phenophases (Cosmulescu et al., 2022) and that can change the quality of accumulated compounds in fruits.

Although bigger fruits seem more appealing to consumers, small blueberries are known to have higher polyphenols, lycopene, and β-carotene than big ones (Hera et al., 2023).

Studies confirm that the amount of sugar contained in blueberries has a high impact on consumers' preferences, especially for children, and that can change the way people choose their fruits (Mennella et al., 2017).

In recent years, the surfaces cultivated with highbush blueberry increased all over the world for their nutritional values, being worldwide recognized as a 'superfruit' (Yan et al., 2023). Being an important source of minerals and antioxidants (flavonoids, polyphenolic compounds, especially anthocyanins) (Diaconeasa et al., 2015), (Okan et al., 2018) with proven effects on human health (Ferrão et al., 2022), these fruits are included in the everyday diet and the demand can influence the prices, the markets, and also the surfaces cultivated with highbush blueberry. Being highly perishable, blueberries have a seasonal availability and is a global race to find new ways

of using these super-fruits so a large number of people benefit from their nutraceutical capacity (Duan et al., 2022).
Traditionally, the fruits' taste has been evaluated by people specialized to distinguish aspects of flavor, texture, and taste through sensorial tests, although this method is expensive and takes time. E-tongue systems (electronic tongue) have been developed in a way that can mimic the mechanism of human taste and they've been successfully used for testing juices, tea, and wine, starting to be recognized as a promising low-cos technique (Zeng et al., 2020).
Even though e-tongue systems have several advantages over human capabilities, not showing fatigue and with a range of multiple substances (Marx et al., 2021), human subjectivity should be taken into consideration by the breeders to breed specific varieties of fruits in answer to consumers' demands.
The present study proposes to assess the consumers' preferences regarding 30 blueberry varieties, 8 of them Romanian varieties, to have a bigger picture of the people's demand in this field.

MATERIALS AND METHODS

The testing session took place on August 4, 2023, and 30 varieties of blueberries were analyzed, from which 8 were Romanian varieties, 'Delicia', 'Vital', 'Lax', 'Prod', 'Augusta', 'Compact' and 'Simultan', and 22 foreign varieties: 'Pink Lemonade', 'Patriot', 'Northland', 'Coville', 'Reka', 'Hortbleu petit', 'Blueray', 'Sunshine blue', 'Legacy', 'Titanium', 'Spartan', Putte', 'Gupton', 'Brigitta', Huron', 'Jersey', 'Ozark blue', 'Hardy blue', 'Draper', 'Denise blue', and 'Bluecrop'.
The indicators used for expressing quality were: size, color, firmness, juiciness, taste, and flavor. The criteria used for evaluation involved grades from 1 to 5; '1' meaning 'I don't like it' (insufficient), and '5' meaning 'I love it' (excellent).
The age of the responders ranged between 11 and 71 years old, with an average of 41, showing a large range age-wise, with a total of 51 accessors.
The graphs were made with the Microsoft Excel software, version 2401.

RESULTS AND DISCUSSIONS

Total scores for the tested varieties revealed that the values are close with small differences between varieties. In the first figure, all varieties were ranked by the highest score after summing all the indicator averages. The highest scores went to 'Brigitta' with 25.6 points out of 30 possible, and Pink Lemonade with 25.1 points. Five different varieties scored 24 points, one of them being a Romanian variety ('Delicia').
The lowest score was acquired by the Romanian varieties 'Lax' and 'Simultan' with only 20.1 and 19.6 points.

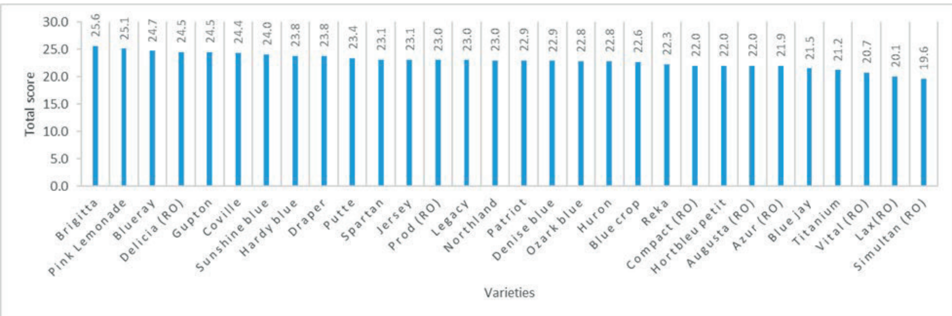

Figure 1. Total scores for the tested varieties. Error bars represent standard error of the mean.

Firmness is an indicator related to blueberry texture and freshness. The highest ranked varieties were 'Brigitta' with 4.6 points, followed by 'Gupton' with 4.3 points. (Figure 2)
The Romanian variety with the best firmness ranking is 'Delicia' with 4.2 points, followed by 'Prod' with 4.0 points.

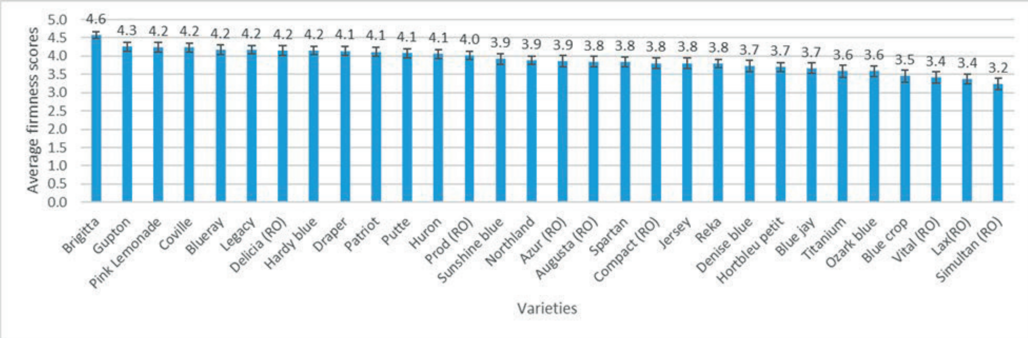

Figure 2. Firmness appreciation. Error bars represent standard error of the mean.

The highest score for juiciness was received by 'Brigitta' with 4.5 points, followed by 'Pink Lemonade' with 4.1 points, and the Romanian variety 'Delicia' with 4.0 points (Figure 3). Another Romanian variety with a good ranking is 'Prod' with 3.8 points.

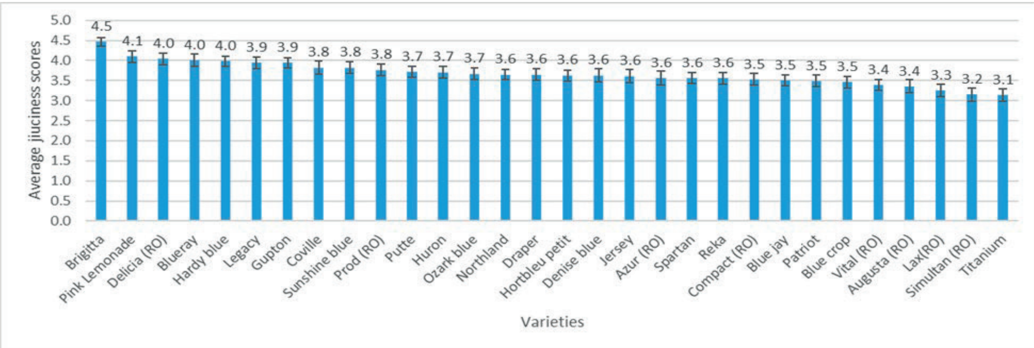

Figure 3. Juiciness appreciation. Error bars represent standard error of the mean.

Taste and flavor are very important traits in a sensorial test and Figures 4 and 5 show that 'Pink Lemonade' was the favorite for both taste and flavor.

With 4.1 points for taste and 4.2 points for flavor 'Pink Lemonade', is the most appreciated variety of blueberry. Both 'Brigitta' and 'Blueray' varieties are also at the top for taste and flavor but with an inversed ranking. The taste was appreciated with 3.9 points for 'Blueray' and 3.8 points for 'Brigitta' and the flavor was appreciated with 4.0 points for 'Brigitta' and 3.9 points for 'Blueray'.

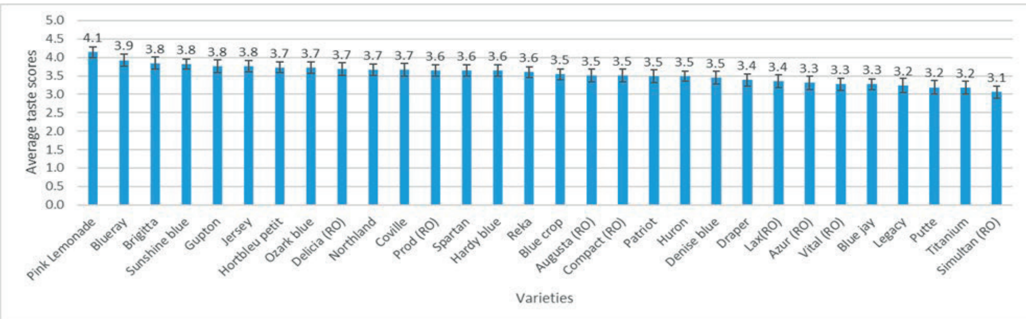

Figure 4. Taste appreciation. Error bars represent standard error of the mean.

The Romanian varieties that impressed with their taste was ‘Delicia’ ranked 3.7 points and ‘Prod’ 3.6 points (Figure 4). As for flavor, ‘Prod’ ranked 3.8 points and ‘Delicia’ 3.6 points, both in top 10 (Figure 5).

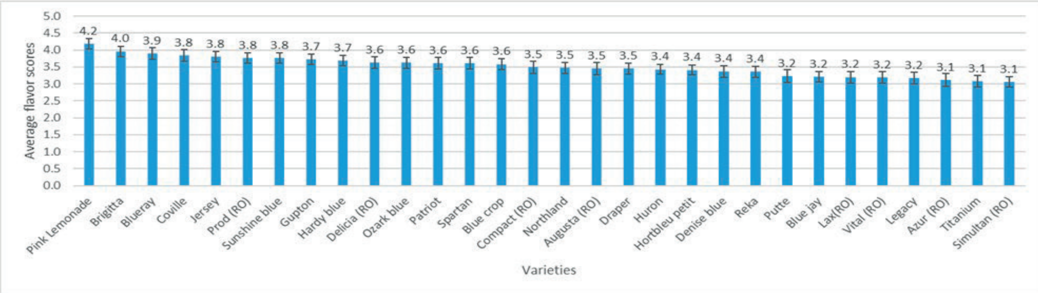

Figure 5. Flavor appreciation. Error bars represent standard error of the mean.

The size and the color of blueberries are also important traits for consumers because the fruits are seen first, and thereafter are bought and tasted. Figure 6 shows that in the sensorial test the best appreciation for size was won by the ‘Putte’ variety with 4.7 points, followed with a small difference, by ‘Draper’. Also ‘Denise blue’ and the Romanian variety ‘Delicia’ scored both 4.5 points.

Other Romanian varieties were appreciated with 4.2 points (‘Azur’) and with 4.1 points (‘Augusta’).

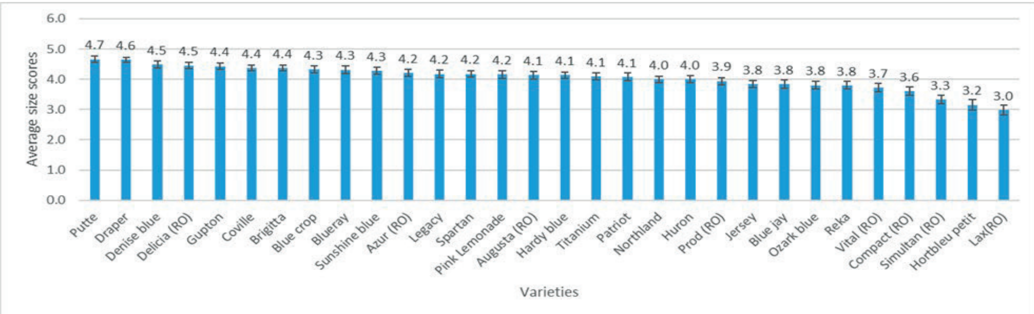

Figure 6. Size appreciation. Error bars represent standard error of the mean.

For the fruits color, the top is reached by ‘Putte’, ‘Delicia’, and ‘Draper’ with the same score – 4.5 points, followed by seven varieties with the same rank, each with 4.4 points (Figure 7). Except ‘Delicia’, no other Romanian variety was on top 10.

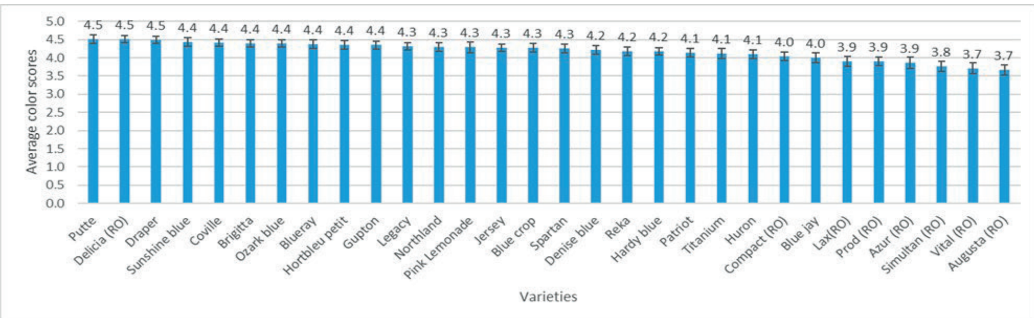

Figure 7. Color appreciation. Error bars represent standard error of the mean.

In 2017, a similar sensorial study was conducted with 26 blueberry varieties (Asănică, 2018) and from those 26 varieties, 15 are also found in the present study. The big surprise was 'Pink Lemonade' variety that had the lowest ranking in 2017 for all indicators, and after 6 years, was the most appreciated for 4 out of 6 indicators (flavor, taste, firmness and juiciness). Also, in 2017 'Delicia' was in top 3 only at firmness, but now was in the top 3 for 3 indicators (color, size and juiciness).

That shows an important evolution of consumer's taste. 'Pink Lemonade' is a variety with a soft pink color but in this study was highly appreciated for taste, flavor, juiciness, and firmness, pointing that at least for the present study, the color, although important, was superseded by the taste and flavor.

CONCLUSIONS

The only Romanian variety that was high ranked in this sensorial study was 'Delicia' reaching the top for 3 out of 6 indicators: juiciness, firmness, color and size.

'Brigitta' had the highest total score at this sensorial test. 'Pink Lemonade' had the highest score at taste and flavor. 'Putte' had the highest score at size and color.

The most overall appreciated varieties of blueberries were 'Brigitta', 'Pink Lemonade', and 'Putte' with high scores for almost all indicators.

Sensorial tests show shifts that can appear in consumers choices. The consumers preferences can change easily in time and these sensorial tests are important for breeders because they can use their results for selecting traits that are important for consumers, improving the fruits variety by using as genetic donor varieties that rank high in consumer preferences, and also are important for farmers, giving them the information they need to cultivate the varieties that are appreciated by consumers.

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AGROPRODUCTIVE EVALUATION OF SOME SWEET CHERRY CULTIVARS IN THE PEDOCLIMATIC CONDITIONS OF N-E ROMANIA

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Abstract

The sweet cherry (Prunus avium L.*) can be one of the most profitable fruit crops grown in temperate climates, highly appreciated worldwide due to the taste, color and nutritional value of the fruits. This study was carried out in the 2021-2023 years using some foreign ('Regina', 'New Star' and 'Kordia') and autochthonous cultivars ('Bucium' and 'Maria') from the Research Station for Fruit Growing (RSFG) Iasi. The aim of this research was to evaluate the productivity in the context of current climate change by determining the main physical and biochemical characteristics of the fruits (weight, diameter, color, sugar composition, titratable acidity and vitamin C), the total fruit production as well as the productivity index related to the trunk section area. The obtained results were interpreted statistically and although there was a variability between the experimental variants, the local cultivars present characteristics that allow agrobiodiversity and a better planning of future improvement programs.*

Key words*: fruit quality, growth, production, Prunus avium* L.

INTRODUCTION

Sweet cherry is an important fruit species that is cultivated and distributed globally (Palasciano et al., 2022). In addition to the traditional and modern known cultivars in Europe, there are also a multitude of cultivars obtained as a result of the genetic improvement of the growing countries, representing a way of completing and improving the current assortment (Iurea et al., 2019). In Romania, the area of sweet cherry orchards being on average on the last 5 years approximately 4 thousand hectares annually, and the fruit production approximately 45 thousand tons (FAO, 2021). Sweet cherries, are among the first fresh fruits of the year and the most appreciated due to its organoleptic characteristics (color, aroma), but also due to recognized benefits for human health (biochemical composition) (Blando & Oomah, 2019). Although the sweet cherry is an economically profitable crop, it also has several characteristics that subject it to multiple risks in the context of current climate change (Lang, 2019; Păltineanu & Chitu, 2020).
In order to limit climatic stress factors (high temperatures, drought) and improve production and its quality, genetically (rootstock) and horticulturally (training systems) research is needed (Whiting et al., 2005).
Regarding the rootstock, the adaptability of *Prunus mahaleb* to the continental climate, tolerance to drought, hot summers due to the root system, expand its importance for sweet cherry culture in the future (Martins et al., 2021; Hrotkó et al., 2023) becoming increasingly used in European countries, in Central Asia and Northwest China (Ercisli et al., 2006; Hrotkó, 2016). These traits gain greater importance due to accelerated climate change and replanting conditions. Although researchers agree that rootstocks affect fruit quality, growth control on mahaleb rootstocks can be managed by applying frequent pruning and maintaining an optimal canopy for an optimal production (Tabakov et al., 2020; Martins et al., 2021).
The recent increase in sweet cherry production worldwide has given new importance to the quality of the fruits. Thus, recent studies emphasize the value of balancing the crop load but also of increasing the dimension and quality of the fruits. For sweet cherries, fruit size and color remain the most important attributes, and

the development of strategies to improve fruit quality through research on rootstocks, cultivars, crop loading, environmental factors and final production is of great interest (Whiting & Ophardt, 2005; Zhang & Whiting, 2011; Malchev et al., 2022).

The objective of this study was to compare the growth, the production yield as well as some physical and biochemical qualities of the fruits of some foreign with autochthonous sweet cherry cultivars, in the context of current climate changes.

MATERIALS AND METHODS

The study was carried out during the years 2021-2023 using five cultivars of sweet cherry with the same decade of fruit ripening, of which three are foreign: ‘Regina’, ‘Kordia’, ‘New Star’ and two Romanian cultivars: ‘Bucium’ and ‘Maria’. The biological material is part of a competitive crop within the Research Station for Fruit Growing (RSFG) Iasi, geographically located in the North-East of Romania (47°20′N and 27°60′E). The cultivars were grafted on rootstock of medium to high vigor *Prunus mahaleb* L., and trained in an improved Spanish vase form. During the study, the orchard was not irrigated and the fertilization and maintenance management was carried out in accordance with the agronomic specifics of the sweet cherry (Quero-García et al., 2017).

The weather conditions monitored in the experimental field during the research years (2021-2023) are shown in Table 1. The average annual temperature of the last three years was 11.3°C, and the total precipitation was 430.3 mm with a deviation of 87.5 mm from the multiannual values.

All determinations were performed on three replicates, from three trees, annually and statistically interpreted by the method of multiple comparisons with the Duncan test. Differences were considered significant at $p < 0.05$ and are indicated by different letters.

The weight was carried out by weighing with the electronical scale 0.01G Radwag type sensitivity and the diameter is the average of two diameters, made by means of a precision digital caliper.

Epidermal color was evaluated using a Minolta colorimeter (CR400 C. Minolta Japan) to determine chromaticity values using the indices: L^* (for lightness), a^* (shades from green to red), and b^* (shades from blue to yellow) (Ibraheem et al., 2012).

The soluble dry solids (SDS) content was measured with a digital refractometer (Hanna Instruments HI96804) and the results expressed in °Brix. Titratable acidity (TA) was determined by acid-base titration with 0.1 N NaOH to the end point of pH 8.1. The results were expressed as mg of malic acid content×$100g^{-1}$ fruit (Hayaloglu & Demir, 2016). For vitamin C content, the 2,6-dichloroindophenol titrimetric method of juice analysis (AOAC Method 967.21) was used and the results were expressed in mg ascorbic acid×100 g^{-1} fruit (Nielsen, 2017).

The cross-sectional area of the trunk, TCSA expressed in cm^2 was calculated by ½ the diameter of the $trunk^2$ × π and the projection volume of the canopy expressed in m^3, CV by [(sum of two diameters of the $crown)^2/2]$+crown height ×0.416 . Cumulative production efficiency index was calculated by relating annual production (kg per tree) to average TCSA (cm^2) and CV (m^3).

Table 1. Climate condition at Iaşi county
(RSFG Iași-Romania, 2021-2023)

Month	Air temperature (°C)			Precipitation (mm)		
	*Av.	M.a	Dev.	Sum	M.a	Dev.
I	1.1	-3.3	+4.4	14.2	28.9	-14.7
II	1.6	-1.5	+3.1	19.1	27.4	-8.3
III	4.5	3.1	+1.4	38.3	28.1	+10.2
IV	9.3	10.3	-1.0	46.5	40.3	+6.2
V	16.0	16.1	-0.1	30.9	52.5	-21.6
VI	20.7	19.4	+1.3	44.7	75.1	-30.4
VII	23.2	21.3	+1.9	58.2	69.2	-11.0
VIII	23.0	20.5	+2.5	68.1	57.6	+10.5
IX	16.7	16.3	+0.4	30.7	40.8	-10.1
X	12.1	10.1	+2.0	14.3	34.4	-20.1
XI	6.6	4.0	+2.6	39.9	34.6	+5.3
XII	1.3	-0.9	+2.2	25.1	28.9	-3.8
Av./Sum	**11.3**	**9.6**	**+1.7**	**430.3**	**517.8**	**-87.5**

* Av.-average; M.a.- Multiannual (1969-2019); Dev.-Deviation

RESULTS AND DISCUSSIONS

The results of the characterization of the morpho-physiological traits are reported in Table 2. The fruit weight of the sweet cherry cultivars during the study period ranged from 7.02 g (‘Maria’) to 10.52 g (‘Regina’), and the largest diameter was highlighted at the ‘New

Star' cultivar (23.63 mm). Although the fruit ripening stage occurs in the same decade, it is influenced by the load of the crop of the year, but also by the genetic character of the cultivars (Kurlus, 2008; Magri et al., 2023).

Table 2. Physical characteristics of fruits (n = 3, RSFG Iași-Romania, 2021-2023)

Cultivar	Weight[1]	Diameter[1]	Colour[1]		
	(g)	(mm)	$\mathscr{L}$	a*	b*
Regina	10.52 a	23.62 a	20.94 a	19.04 a	2.29 a
Kordia	9.28 b	21.58 b	20.15 b	15.91 b	0.94 b
New Star	8.48 c	23.63 a	19.42 b	15.56 b	1.14 b
Bucium	8.40 c	22.57 ab	21.23 a	19.17 a	2.27 a
Maria	7.02 d	21.8 b	21.23 a	15.30 b	2.63 a

[1]Different letters after the number correspond with statistically significant differences for p 5% - Duncan test.

Fruit weight and fruit dimensions in diameter (width, thickness and length) are very important properties being the parameters that give the appropriate commercial appearance to the fruits. Sweet cherry cultivars with large fruits (both in size and weight) are increasingly valuable, but these parameters are strongly influenced by the climatic conditions and the applied culture system (Sirbu et al., 2018), being able to vary from year to year even by 4 g fruit weight (Hayaloglu & Demir, 2016).

Besides size, another key criterion used to evaluate the attractiveness of sweet cherries is fruit color (Romano et al., 2006). The color of the epidermis is considered to be the most significant indicator of the quality and maturity of cherries (Magri et al., 2023). The fruit color parameters of the evaluated cultivars are reported in Table 2. Luminance values ($L*$) ranged from 19.42 at 'New Star' to 21.23 at local cultivars 'Bucium' and 'Maria'. The shades of intense red ($a*$) with the highest values were found on average in the cultivar 'Bucium' (19.17).

Important biochemical components such as dry soluble solids (SDS), acidity as well as vitamin C influence consumer preference for the nutritional quality of fruits (Serradilla, 2012) and are presented in the Table 3.

Although the chemical composition varied annually, on average over the course of the study, the lowest SDS content was recorded at 'Kordia' cultivar (19.8°Brix), while the highest was at 'Bucium' (22.5°Brix), followed by 'Maria' (21.4°Brix) and 'Regina' (20.93°Brix). Depending on the cultivar of sweet cherry, the SDS content of fruits during maturity, according to numerous researches (Girard & Kopp, 1998; Guarino at al., 2010), can have minimum values of 13.2°Brix and can reach maximum values of 25.5°Brix. Similar findings to our results were also found in Wen et al. (2014), which showed SDS values between 17.77 and 19.97°Brix, in medium ripening cultivars.

TA estimates for different cultivars of sweet cherry is usually by malic acid content (Ricardo-Rodrigues et al., 2022), ranging from 0.62 mg malic acid/100 g for 'Kordia' to 1.03 mg malic acid/100 g for 'Bucium' cultivar. Significant differences were observed between all the variants examined (Table 3).

In our study, the highest level of vitamin C was found in the cultivar Bucium (23.7 mg×100 g^{-1} F.W.), followed by 'Kordia (20.17 mg×100 g^{-1} F.W.) and 'New Star' (20.0 mg×100 g^{-1} F.W.). The rate of formation of the vitamin C background in medium-ripening sweet cherry fruits exceeds the average parameters of the cultivars (Ivanova et al., 2022), a fact also confirmed in this study, where the vitamin C content was maximum.

Table 3. Chemical characteristics of fruits (n = 3, RSFG Iași-Romania, 2021-2023)

Cultivar	SDS[2]	TA[3]	Vitamin C
	(°Brix)[1]	(mg /100 g^{-1})[1]	(mg·100 g^{-1})[1]
Regina	20.93 bc	0.68 cd	18.00 b
Kordia	19.80 c	0.62 d	20.17 ab
New Star	19.83 c	0.71 c	20.00 ab
Bucium	22.53 a	1.03 a	23.67 a
Maria	21.40 a	0.88 b	18.33 b

[1]Different letters after the number correspond with statistically significant differences for p 5% - Duncan test.
[2]SDS- Soluble Dry Solids
[3]TA- Total Acidity

Vitamin C depends on weather conditions the growing season as well as the orchard area (Hayaloglu & Demir, 2016) thus, the same cultivar, analyzed in different surveys can have very significant variations. Previous data of the 'Regina' cultivar showed a minimum vitamin C content of even 7.29 mg×100 g^{-1} F.W. and in the conditions of N-E Romania, in the 3 years it recorded a value of 18.0 mg×100 g^{-1} F.W. Other research (Średnicka-Tober et al., 2019), indicates that sweet cherry fruits can reach a significantly higher content of vitamin C, up to 42.89 mg×100 g^{-1} F.W.

An analysis of tree productivity, both on average per tree (kg) and per surface unit (t/ha) was presented in Table 4. Thus, among the analyzed cultivars, production with values greater than 30 kg per tree was registered at ‘Bucium’ (34.4 kg) and ‘Kordia’ (31.2 kg).

The drought conditions during the years of the study influenced the growth and productivity of the studied cultivars differently. Thus, depending on the TCSA and CV of the trees, the productivity index of the sweet cherry trees was also calculated.

The obtained results confirmed that ‘Bucium’ produced a significantly higher production yield compared to the other cultivars, although TCSA and CV had medium values.

The greatest vigor of trunk growth was recorded in the ‘Regina’ cultivar (148.3 cm^2) and in crown volume, ‘New Star’ (8.93 m^3). The ‘Maria’ cultivar stood out with the lowest growth vigor, but with satisfactory results in terms of the productivity index (0.29 kg/cm^2 and 3.46 kg/m^3).

Table 4. Productivity and vigor of the studied sweet cherry trees (n = 3, RSFG Iași-Romania, 2021-2023)

Cultivar	Production		TCSA[2]	CV[3]	Productivity index	
	(kg/tree)[1]	(t/ha)[1]	(cm^2)[1]	(m^3)[1]	(kg/cm^2)[1]	(kg/m^3)[1]
Regina	22.53 [b]	11.27 [b]	148.33 [a]	8.05 [b]	0.15 [c]	2.80 [b]
Kordia	31.20 [a]	15.60 [a]	125.82 [c]	7.22 [d]	0.25 [b]	4.32 [a]
New Star	24.67 [b]	12.33 [b]	135.94 [b]	8.93 [a]	0.18 [c]	2.76 [b]
Bucium	34.43 [a]	17.22 [a]	100.28 [d]	7.55 [c]	0.34 [a]	4.56 [a]
Maria	25.83 [b]	12.92 [b]	88.33 [e]	7.47 [c]	0.29 [b]	3.46 [b]

[1] Different letters after the number correspond with statistically significant differences for *p* 5% - Duncan test;
[2] TCSA - Trunk cross-sectional area;
[3] CV – Crown volume;

To determine the relationship between two variables, the correlation coefficient between average fruit weight and production, TCSA, CV and productivity index (kg/cm^2) was calculated (Table 5).

Table 5. Pearson correlation coefficient (r) between mean fruit weight and productivity and vigor indices (RSFG Iași-Romania, 2021-2023)

Correlation coefficient	Weight[1] (g)
Production (kg/tree)	-0.219
TCSA[1] (cm^2)	0.863
CV[2] (m^3)	0.139
Productivity index (kg/cm^2)	-0.650

[1] TCSA - Trunk cross-sectional area;
[2] CV – Crown volume;

Thus, in the relation fruit weight-production (r=-0.219) and weight-crown volume (r=0.139), the correlation coefficient was weak, having values ≤0.35.

In the relationship weight-TCSA (r=0.863) and weight-productivity index (r=-0650), the correlation was significantly high.

The statistical interpretation of the correlation coefficient was in agreement with Taylor, 1990.

CONCLUSIONS

The studied cultivars are of high productivity, and o superior fruit quality, finding favorable growth and productivity conditions in the North-Eastern part of Romania.

Although there were conditions of water and heat stress, the vigorous rootstock *Prunus mahaleb* imposed great resistance on both local and foreign cultivars.

The autochthonous cultivars created and approved within the Research Station for Fruit Growing (RSFG) Iasi, ‘Bucium’ and ‘Maria’ can be successfully introduced into the international assortment of mid-ripening sweet cherry cultivars, possessing physical and chemical qualities similar to or even superior to internationally relevant cultivars as well as a very good productivity.

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A REVIEW OF BLACKCURRANT CULTURE TECHNOLOGY

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Abstract

As consumers increasingly prioritize health and wellness, the nutritional profile of blackcurrants, with its mix of vitamins, antioxidants, and anti-inflammatory properties, positions them as a valuable addition to a balanced and nutrient-rich diet. Blackcurrants are valued for multiple reasons, with distinct attributes that contribute significantly to their prominence. They are recognized for early fruit-bearing, consistently high yields, and adaptability to diverse climatic and soil conditions. These attributes enhance the economic viability of cultivating blackcurrants, contributing to their widespread popularity among fruit growers. This review aims to provide a screening of the cultivation technologies for the blackcurrant crop, focusing on specific characteristics such as planting, soil management, fertilization, irrigation, pruning, disease and pest management. This paper can be a useful tool for anyone interested in blackcurrant crop technology.

***Key words**: disease, fertilization, irrigation, pruning, soil management.*

INTRODUCTION

The blackcurrant, also known as *Ribes nigrum*, is a medium-sized woody shrub that thrives in colder climates, due to its ability to withstand harsh winters, and is valued for its juicy and aromatic berries (Woznicki et al., 2016), although, in certain regions, blackcurrants are grown specifically for their buds, from which a valuable essential oil can be extracted. Due to its high cost, this oil is typically reserved for use in luxury perfumes (De Toro, 1994). The small fruits of the blackcurrant are utilized fresh, can be preserved by freezing, and serve as a valuable raw material for the food and confectionery industries, utilized in the production of juices, wines, jams, jellies, teas, and various other food products and ingredients (Sazonova, 2015; Mattila et al., 2016; Sazonov et al., 2020).

Blackcurrants are relatively easy to propagate and cultivate, with considerations of economic viability also playing a crucial role in these decisions (De Toro, 1994).

Researchers and farmers have crafted precise production systems for numerous temperate horticultural crops worldwide. These systems excel in utilizing labor and chemical inputs effectively. They allow for crops to be cultivated over extended seasons and stored for prolonged periods. Cultivars tailored to particular growing regions have been bred, and pest management strategies have been seamlessly integrated into production methods. Farmers have experienced enhanced efficiency, at least in the short term. Meanwhile, consumers have enjoyed long-term advantages such as reduced prices and a consistent supply (Pritts, 2002).

Furthermore, to advance crop quality and performance, numerous blackcurrant breeding programs have been established across various research institutions globally. These breeding initiatives are widespread and conducted in countries such as New Zealand, Denmark, France, Germany, Japan, the Netherlands, Norway, Poland, Ukraine, the United Kingdom, the United States, Finland, Canada, Sweden, Estonia, Latvia, Lithuania, Romania, Russia, and Serbia. Each of these nations is dedicated to improving blackcurrant varieties through selective breeding methods, focusing on enhancing traits such as yield, resistance to key pests and diseases, and fruit quality suitable for processing, freezing, and fresh markets (Cortez et al., 2019). Moreover, there is an emphasis on developing cultivars adapted to local soil and weather conditions, as well as mechanical fruit harvesting (Pluta and Zurawicz, 2002b).

This study aims to offer an overview of cultivation techniques for blackcurrant crops.

MATERIALS AND METHODS

This review examines the current state of cultivation technologies for blackcurrant crops, covering key aspects such as planting, soil management, fertilization, irrigation, pruning, and disease and pest management. The goal is to offer insights into the current state of cultivation practices for blackcurrant crops.

RESULTS AND DISCUSSIONS

Planting

Blackcurrant cultivation occupies a relatively modest geographical area, yet it is a crop of considerable economic importance, being increasingly acknowledged for its rich content of vitamin C and anthocyanins (Vagiri et al., 2012). Particularly in Europe, the primary region for blackcurrant cultivation, production is on the rise, and there is a growing interest in extending cultivation to regions and countries where *R. nigrum* is not presently cultivated (Mitchell et al., 2011). Blackcurrants are relatively straightforward to grow, thriving once they've taken root, easily outcompeting weeds. Typically, they're planted in either autumn or spring. Being deciduous, their growth cycle is mainly regulated by light and temperature. Although they endure low temperatures well during dormancy, they're sensitive to spring frosts, particularly during flowering. Yet, some varieties boast genetic resilience against frost damage. If the aim is to prioritize vigorous vegetative growth over fruit production, the risk of frost damage decreases (De Toro, 1994). Blackcurrant has a relatively high chilling requirement and is one of the fruit crops that is potentially at risk in parts of Europe due to the lack of winter chilling forecast in projections of future climatic conditions (Atkinson et al., 2013; Jones et al., 2012). During winter, the buds of numerous temperate woody plants, such as blackcurrant, experience a dormancy cycle to avoid premature bud bursts during brief periods of warmer weather and the ensuing damage to shoots as colder conditions return. This cycle is triggered by a reduction in day length and/or a decrease in temperature (Heide, 1974; Fennell and Hoover, 1991; Olsen, 2010).

The timing of phenological events is crucial for perennial fruit crops, particularly in selecting suitable cultivars for specific regions. If a cultivar fails to respond adequately to environmental conditions, it can result in significant consequences such as reduced annual growth, lower fruit yield, and even plant mortality (Chung et al., 2013).

Cultivating blackcurrants requires substantial labor and resource investment. Establishing and maintaining a blackcurrant crop demands significant time and energy, both human and mechanical. Employing mechanized methods notably decreases the time and effort needed for diverse agricultural tasks, thereby crucially enhancing efficiency and profitability for farmers (Yläranta and Ryynänen, 1981).

Before planting, soil preparation is crucial to eradicate perennial weeds (Karlsson, 1985). Traditionally, blackcurrants are spaced 0.5 to 2.0 meters apart between plants and 2.4 to 4.0 meters apart between rows, facilitating mechanical weed control (De Toro, 1994). This spacing also needs to ensure the formation of compact rows, preventing gaps between plants or overlap of branches among adjacent plants (in cases of insufficient spacing between plants within a row) (Asănică, 2017).In a study conducted by Rousseau and Roy in 2002, different plant spacings of 55, 70, 85, and 100 cm were compared for both cultivars 'Ben Alder' and 'Ben Lomond'. Results showed that for 'Ben Alder', the yield per bush remained consistent across different plant spacings, while for 'Ben Lomond', the yield improved with increased plant spacing. However, the mean weights of fruit did not show significant variation among these spacings, with 'Ben Alder' averaging 105 g and 'Ben Lomond' averaging 163 g. While studies indicate higher fruit and wood production per unit area with increased plant density, the economic implications of denser plantings must be considered. Although the literature suggests that the highest wood and bud yields occur at very high plant densities, this entails significant establishment costs. Therefore, under commercial conditions, it may be wiser to accept lower yields to minimize plantation costs and enhance long-term profitability (De Toro, 1994).

Soil Management

The importance of soil cultivation in blackcurrant crops is to ensure adequate water and air penetration at the root system level. It involves activities such as weed control, soil aeration, and refining to maintain suitable soil moisture conditions for the plants. These tasks begin immediately after planting and continue throughout the growing season to encourage healthy growth and minimize weed competition. Similar to many other fruit crops, blackcurrants thrive in soils with a favorable texture, such as loam or clay loam. Sandy soils typically have limited water retention capacity, while heavy clay soils can impede the development of their delicate root system (Karlsson, 1985).

Special care should be taken to ensure that tools are used at an appropriate depth to avoid damaging the roots. The number of hoeing per row is typically reduced to 1-2 per year when herbicide application is also employed (Mladin and Mladin, 1992).

Between the rows, the soil can be maintained as bare soil or as grassed (lawn), with regular mowing of the grass. Along the row, mulching can be applied, covering a width of 1.0-1.3 meters with materials like straw, grass, and green plants. Periodically, usually every 2-3 years, the mulch is incorporated into the soil in autumn through deep plowing, enriching the soil with organic matter. Additionally, mulch helps retain moisture in the soil, improve its structure, and delay weed emergence. If possible, black plastic film (P.V.C.) can also be used for soil mulching (Mladin and Mladin, 1992).

Larsson et al. (1997) conducted a study investigating soil microbial parameters in blackcurrant fields under different mulches and cover crops. They found that wood chip mulching boosted basal respiration rates but caused nitrogen deficiency in the bushes despite the initial nitrogen supply. This highlights the importance of assessing microbial parameters for sustainable growing systems and identifying environmental stressors.

In a study by Paunović et al. (2017), three soil management systems (continuous tillage, sawdust mulch, and black polyethylene foil mulch) were compared alongside four blackcurrant cultivars ('Ben Lomond', 'Titania', 'Čačanska crna', and 'Tiben'). Soil management system x cultivar Interactions were noted between soil management systems and cultivars regarding generative potential and physical properties of the cluster and fruit. However, no significant interactions were observed for fruit chemical traits, except for soluble solids content. In another investigation, Paunović et al. (2020) assessed the effects of sawdust and black foil mulches on soil properties, growth, and yield of blackcurrant (*Ribes nigrum* L.) over three years, including a bare soil control group. Sawdust mulch notably increased nutrient content and microbial activity while moderating soil temperature and moisture levels, ultimately leading to improved growth and yield of blackcurrants compared to bare soil and foil mulch treatments.

Additionally, Laugale et al. (2019) conducted a study in Latvia comparing two growing practices: one involving the application of pine bark mulch and the other without mulching, where herbicides were applied in rows across seven blackcurrant cultivars. The study found no significant differences in blackcurrant phenological development, productivity, or fruit size between the two growing practices. However, the use of pine bark mulch led to reduced weed growth and less damage from leaf spot diseases and aphids compared to growing without mulch.

The adequate growth of root systems and the dynamics within the rhizosphere, involving symbiotic mycorrhizal fungi and rhizosphere bacteria, are crucial for the healthy development of plants in both natural environments and agricultural settings such as orchards and berry fruit plantations (Sas-Paszt and Mercik, 2004; Sas-Paszt and Żurawicz, 2004, 2005). In a 2013 study led by Derkowska and colleagues, the impact of mycorrhization and mulching on arbuscular mycorrhizal fungi colonization in 'Ojebyn' and 'Tiben' blackcurrant bushes was investigated. Root samples were collected from various experimental combinations (control, peat substrate, bark, sawdust, manure, compost, mycorrhizal substrate, and straw) to assess mycorrhizal frequency. The results indicated that the highest values of mycorrhizal frequency and intensity were recorded in the roots of 'Tiben' bushes inoculated with the mycorrhizal substrate (F = 37.78%, M = 0.38%) and those mulched with sawdust (F = 21.11%, M = 0.21%).

Fertilization

In modern, environmentally friendly farming, it's important to optimize nutrient uptake from both organic (like natural mulches) and organo-mineral fertilizers while minimizing mineral fertilizer and chemical pesticide use to maintain ecological balance. To achieve high yields sustainably, traditional intensive farming methods, relying heavily on mineral fertilizers and pesticides, can be replaced with organic practices like using manure, straw, and natural bioproducts such as biofertilizers and composts enriched with beneficial microorganisms (Mladin and Mladin, 1992). Research on organic farming methods aims to produce high-quality crops while safeguarding the environment and human health (Derkowska et al., 2013).

Natural mulches serve as a mineral source (including C, N, and P) for plants following the mineralization of organic matter, with soil macro- and microorganisms deriving energy and essential minerals from organic matter, thus enhancing soil biological activity (Esperschütz et al., 2007). Moreover, humus compounds in mulches significantly affect crop physiology, influencing processes such as water balance, respiration, and photosynthesis (Ngosong et al., 2010).

Although blackcurrants are less demanding in soil fertilization, systematic application of organic and chemical fertilizers leads to large and consistent fruit yields of exceptional quality year after year, significantly extending the economic exploitation period of plantations. Fertilizers are applied judiciously based on the soil's nutrient content and the plants' nutritional requirements. Like other fruit shrub species, blackcurrants require higher potassium and phosphorus levels from the beginning of flowering until fruit ripening, while nitrogen is most critical in the early stages of vegetation for plant growth (De Toro, 1994).

The enhancement of depleted humus and nutrient levels in the soil involves incorporating manure along with variable amounts of nitrogen, phosphorus, and potassium, adjusted according to the soil's nutrient composition. Research has established fertilization ratios for different species and groups of species, with a specific N:P:K ratio of 2:1:3 recommended for blackcurrants. The choice of fertilizer depends on the soil's pH, with acidic fertilizers like ammonium sulfate, superphosphate, and potassium sulfate used for alkaline soils, and basic fertilizers preferred for acidic soils. Manure and less soluble fertilizers are applied in autumn and incorporated into the soil through plowing (Mladin and Mladin, 1992).

Chemical fertilizers can be applied singly or in various mixtures, and nitrogen-based fertilizers are typically applied at the start of vegetation in March. A second fertilization may be done in June if nitrogen deficiency is observed, with nitrogen fertilizers spread evenly along rows or around plants and incorporated into the soil (Mladin and Mladin, 1992).

In recent times, there has been an increasing focus on enhancing plant nutrition through foliar fertilizers. This method is viewed as a promising and environmentally friendly approach to boost crop yield and nutrient utilization efficiency. The targeted application of foliar fertilizer tailored to each variety holds significant promise for maximizing specific yield potential and overall profitability (Vâtcă et al., 2020a).

Foliar fertilizers containing macro and micronutrients are used during spraying against diseases and pests, typically before flowering, 1-2 weeks after flowering, and/or during fruit growth and maturation. These fertilizers address deficiencies observed in plant leaves and other parts, providing nutrients like boron, zinc, iron, and manganese (Mladin and Mladin, 1992). In a recent study, Vâtcă et al., (2020b) tested the specific reaction of three blackcurrant varieties to foliar fertilizers to obtain two growth models. The conclusion was that each variety has a specific response to fertilizers.

Soil cultivation and nitrogen provision are crucial aspects of organic farming, especially when organic fertilizers are required. However, obtaining organic manure from other organic farms can be difficult in practice. Consequently, cover crops are seen as a practical alternative nitrogen source, providing a potential option for organic fertilization on farms focusing on organic blackcurrant production (Lindhart, 2002).

Lindhart (2002) used various mulching methods in an unsprayed field trial to assess their impact on organic blackcurrant production about soil cultivation and nitrogen supply. Mechanical weed control was performed along the bush rows, and cover crops such as vetch (*Vicia sativa*), rye (*Secale cereale*), perennial white clover (*Trifolium repens*), and ryegrass (*Lolium perenne*) were utilized in the trial. Throughout the experimental process, each of the cover crops supplied an adequate amount of nitrogen.

A study by Polunina et al. (2023) investigated the impact of fertilization and plant care on the productivity of a particular blackcurrant variety. The findings demonstrated that optimal conditions, including mineral fertilization, Riverm 5% foliar application, clean fallow, and straw mulching, contributed to improved cluster development. Additionally, combining Riverm 5% foliar application with mineral fertilization resulted in increased blackcurrant yield while Medelyaeva et al. (2023) conducted a study in the Tambov region on leached chernozems, examining the impact of foliar feeding with Aquarium 6 at a concentration of 0.5%, trace elements at a concentration of 0.1%, and urea at a concentration of 1% on the spread of pests and diseases in blackcurrant plantings. The findings indicate a positive effect of these treatments on reducing the number of kidney mites and the development of powdery mildew compared to the control group.

Irrigation

In its natural environment, the blackcurrant plant thrives as an understory shrub, often found beneath taller trees or in partially shaded sections of forests. This habitat offers the plant partial shade and sufficient moisture. The findings indicate that blackcurrant plants inherently possess a low tolerance to drought conditions (Woznicki et al., 2016). In regions with insufficient rainfall, irrigation becomes essential for successful crop growth. The frequency of irrigation sessions varies based on factors like resource availability, soil moisture levels, root system development, growth stages, and specific requirements of the plant species. For blackcurrants, maintaining soil moisture levels at 70-80% throughout the growing season is crucial for optimal growth and fruiting. Additional watering is required at key stages such as inflorescence emergence, end of flowering, start of fruit development, fruit ripening, and post-harvest irrigation to support bud formation and winter preparation.

In the context of blackcurrant cultivation, irrigation technology encompasses the precise application of water, both in terms of quantity and timing, utilizing sophisticated systems and techniques. This approach is crucial for ensuring the optimal growth and development of blackcurrant plants throughout their lifecycle. Irrigation methods can vary depending on resource availability, with options including sprinkler, micro-sprinkler, or drip systems tailored to the specific needs of the crop (Mladin and Mladin, 1992).

Advanced irrigation systems, such as drip irrigation or micro-sprinklers, are commonly employed in blackcurrant cultivation to deliver water directly to the root zone of plants. This targeted application minimizes water wastage through evaporation or runoff and promotes efficient water uptake by the plants. They also enable precise control over the application of water. This level of control is instrumental in mitigating the risks associated with over- or under-watering, which can have detrimental effects on plant health and yield.

In 2002, Rolbiecki et al. published a study aimed at evaluating the effects of drip and micro-sprinkler irrigation on the growth and yield of blackcurrants in light soil. Their findings highlighted the critical role of water in determining yield and quality, with irrigation significantly improving growth and berry production. The study conclusively demonstrated that successful blackcurrant cultivation in such soil and climate conditions requires irrigation.

Trickle irrigation systems have evolved and become available in various types since their introduction. Although these systems find

applications in diverse crops, they are notably advantageous and frequently employed in perennial crops. This assertion is corroborated by studies conducted by Karmeli and Keller in 1975, as well as by Nes et al. in 2002.
Additionally, high-density plantations typically have greater water requirements compared to those with normal density (De Toro, 1994).
One advancement in irrigation technology is the use of soil moisture sensors, which provide real-time data on soil moisture levels, allowing farmers to adjust irrigation schedules accordingly to prevent water stress and avoid overwatering. Modern irrigation controllers can also utilize weather data to automatically adjust irrigation schedules based on local climate conditions, further optimizing water usage (Muneeb et al., 2023).
Fertigation technology, where fertilizers are injected directly into the irrigation system, has become increasingly common in blackcurrant cultivation. This method ensures that plants receive essential nutrients directly at the root level, minimizing waste and runoff while promoting efficient nutrient utilization and environmental sustainability (Muneeb et al., 2023).
The potential of implementing automation through an Internet of Things (IoT) system to enhance traditional surface irrigation systems. Automated systems can operate with minimal manual intervention, utilizing timers, sensors, computers, or mechanical devices to streamline functionality and address issues such as labor demands and water application inefficiencies.
Numerous studies have indicated that implementing automation in irrigation projects, utilizing advanced technologies like intelligent irrigation controllers and wireless sensor networks, can result in substantial water savings, potentially up to 38% (Al-Ghobari et al., 2017; Bowlekar et al., 2019). Various types of soil moisture sensors including tensiometers, gypsum blocks, granular matrix sensors, time-domain reflectometers, and dielectric probes, are available for measuring soil moisture levels, which can be used manually or integrated into automatic irrigation control systems via an IoT system to optimize water application. (Bowlekar et al., 2019; Hardie, 2020; Vera et al., 2021; Pramanik et al., 2022). The sensors detect the advancement of water and provide a signal to halt the flow accordingly.

Pruning

Blackcurrant belongs to the group of fruit shrubs composed of woody plant species that form numerous stems of varying vigor, originating from the root collar area, and giving the appearance of a more or less compact bush. The classic training system for fruit shrubs typically involves using bush-type training forms, which do not require support or trellising of the plant growth. This system has the advantage of lower establishment and maintenance costs (Asănică, 2017).
New crown designs have been implemented for fruit bushes to meet the demands of intensification and enhance fruit quality. The development of these new crown structures represents a significant advancement in modern horticulture. In this regard, Stanislav Strbac applied and tested the vertical cordon training system for black and red currants in Norway (Asănică, 2017).
The first 2-3 years after establishing the plantations involve a series of activities focused on promoting rapid plant development and achieving early yields.
Increased production during the initial years of cropping could potentially be achieved through the implementation of management practices that influence the quantity and length of shoots generated in the first season (Rhodes, 1986).
To facilitate maintenance and harvesting, a flattened bush form is adopted for the plants.
Various activities are carried out during plant management, aligned with the chosen cultivation approach and the biological needs of the fruit bushes to ensure proper growth and fruit production. Pruning systems include formation pruning, fruiting pruning, and rejuvenation pruning.
a) ***Formation pruning***: For species grown as bushes, formation pruning begins at planting by shortening stems to 10-12 cm. After the first year, 3-5 growths are stopped at each plant and shortened to 30-40 cm. After three years, in autumn or spring, 8-14 growths of different ages are left at each bush, eliminating weak, thin, broken, or diseased ones. By the fourth year, the bush is considered formed, consisting of 15-20 stems of varying ages, well-positioned

to allow light penetration and facilitate soil maintenance.

b) ***Fruiting pruning***: Conducted during the fruiting period, it aims to maintain a balance between growth and fruiting processes. Aged stems (over 4-5 years old) are removed and replaced with new growth, while small growth stems, broken or diseased branches and those growing towards the row interval are suppressed. Balancing young and old wood is essential for optimal production.

c) ***Regeneration pruning***: Applied when plants decline to prolong their economic fruiting lifespan. For bush-shaped plants, all stems over one year old at the base are removed, and the remaining stems are pruned to 4-6 buds. Weak growth and poorly positioned stems within the bush are eliminated. Fertilization with farmyard manure, superphosphate, and potassium salt is recommended along with regeneration pruning. Nitrogen-based fertilizers are applied during the vegetation period of the following year. Pruning is done using sharp tree pruning shears and wounds larger than 23 cm in diameter are coated with mastic or oil-based paint to promote rapid healing (Mladin and Mladin, 1992).

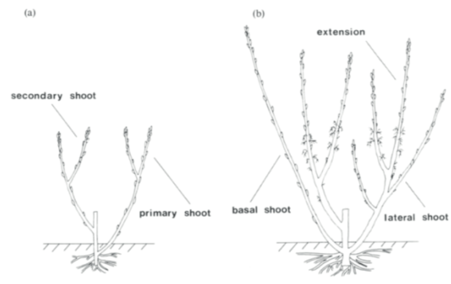
Song et al. (2000) recommend a specific pruning regimen for blackberry plants. Initially, after planting, they suggest cutting the shoots at the base, leaving 5-7 buds untouched. In the second spring 4-5 shoots should be headed back, leaving 25% of the length of the shoot. Finally, after the third year of growth, it advises thinning out the old fruiting branches to maintain plant health and encourage new growth. A study conducted in Lithuania by Sasnauskas and Buskienė (2006), examined the effects of pruning on four blackcurrant cultivars. Pruning induced shoot growth and significantly boosted growth vigor by 40-70% compared to unpruned controls. Pruning at a height of 15-20 cm above the soil led to a yield increase of 16-50% compared to unpruned controls. The average fruit weight after pruning increased by 30%.

To reduce the time spent on maintenance pruning, one effective option is to utilize various pruning devices, as demonstrated in the study by Dalman in 1993. In this study, a pruning device mounted on the side of a tractor for removing bending branches significantly decreased the need for manual pruning with hand tools. Furthermore, it was found that the need for manual pruning was minimal when mechanical pruning was performed every autumn, and old and injured branches were pruned by hand every third autumn. These findings suggest that regular use of mechanical pruning devices can significantly reduce manual effort and streamline the maintenance process in blackcurrant plantations (Dalman, 1993).

Rhodes (1986), conducted a comprehensive three-year study in Canterbury, focusing on the growth and fruit yield of blackcurrant plants propagated from unrooted cuttings. During the first season, buds sprouted into primary shoots, some of which developed into secondary shoots (Figure 1a).

In the subsequent season, both primary and secondary shoots bore fruit, leading to their classification as primary and secondary canes. This period witnessed the extension of these canes and the emergence of lateral shoots from axillary buds, alongside the appearance of basal shoots (Figure 1b).

Moving into the third season, shoots from the previous year bore fruit, now classified as extension, lateral, and basal canes. The growth patterns observed were akin to those witnessed in the second season.



Source: Rhodes, 1986

Figure 1. Blackcurrant plant at (a) the end of the first season after planting, and (b) the end of the second season, after the first harvest of fruit

Disease and Pest Management

Several pests affecting blackcurrants are caused by insects, fungi, or viruses. Around 14 different viruses or virus-like diseases have been reported in *Ribes* species worldwide (Jones, 1992; 1995a). Some of these are confined to particular regions and appear sporadically in crops or limited areas. Among them, Blackcurrant Reversion Disease (BRD) and its vector gall mite (*Cecidophyopsis ribis*) are the most serious pests threatening blackcurrant production (Pluta et al., 2002a), particularly noteworthy for their widespread occurrence and economic impact. BRD is prevalent worldwide, where blackcurrants are grown commercially (Bremer, 1983; Ravkin and Chertovski, 1989; Wood, 1991; Trajkovski and Anderson, 1992; Jones 1992; 2002). The disease has been known for nearly a century. However, the causal virus was identified only a few years ago (Jones et al., 1995b; Lemmetty et al., 1997, 1998).

The symptoms include changes in the appearance of leaves and flowers, as well as a reduction in flower fertility, which can lead to significant losses in production. Due to the significant damage caused by the gall mite vector, which induces galling and sterility of buds on blackcurrant plants (Adams and Thresh, 1987), cultivation of blackcurrants has completely ceased in certain regions of New Zealand and Europe (Jones, 2002)

Gooseberry Vein Banding Disease (GVBD) is another widespread issue, affecting various *Ribes* species and cultivars worldwide, representing a significant virus-like disease in these plants. It is considered the second most prominent virus-like disease, with Blackcurrant Reversion Disease (BRD) being the most significant. GVBD is characterized by noticeable chlorosis along the leaf lamina near the main veins in *Ribes* plants. Additionally, it is known to lead to substantial decreases in plant vigor and yield. This viral disease is likely transmitted by aphids and results in significant declines in plant growth and yield. Control measures include using healthy planting material, removing infected plants, and managing aphid populations to prevent disease spread (Jones et al., 2001).

The identification and characterization of viruses and the development of a sensitive PCR assay have enabled detection within 2 days, making it useful for plant introduction and quarantine programs. This technology also offers new opportunities to study epidemiology, virus-vector relationships, resistance identification in *Ribes* germplasm, and inheritance mode, potentially improving crop control measures (Jones, 2002).

Insects such as slowbugs (*Plesiocoris rugicollis*), hymenoptera (*Pachynematus pumilio*), moths (*Euhyponomeutoides albithoracellus*), *Lampronia capitella*, *Synanthedon tipuliformis*, *Resseliella ribis*, and the aphid (*Hyperomyzus lactucae*), which overwinter in various parts of the plant, are expected to have their life cycles interrupted by annual shoot-cutting.

However, pests and viruses spread by mites are not easily controlled by yearly shoot-cutting. Fungi, whose spores overwinter on fallen leaves or other plant debris, present a different challenge. Fungi may proliferate under favorable conditions, especially in high-density and well-fertilized cultivation. *Sphaerotheca mors-uvae*, causing powdery mildew, is a significant fungal disease, thriving in dry weather; its spores overwinter in fallen leaves and buds, though some varieties exhibit resistance.

Blackcurrants may exhibit susceptibility to white pine blister rust (*Cronartium ribicola*) and leaf spot infections (*Gloeosporidiella ribis*), which occur annually and can lead to significant leaf drop by September. Additionally, severe infections of American gooseberry mildew (*Sphaerotheca mors-uvae*) can adversely affect yields, resulting in minimal shoot growth. (Lindhart, 2002)

Other fungi such as leaf spot (*Septoria ribis*), *Puccinia caricina*, and grey mold (*Botrytis cinerea*) also pose challenges, particularly in low-density cultivation. *Septoria ribis* infections are prevalent among currant species (Parikka et al., 2002). These diseases are typically managed through chemical control methods in spring, involving one spray before flowering and another within a week after flowering (Tapio, 1972). The interval between sprays may vary, with several weeks between applications depending on weather conditions, although later treatments are not recommended (Parikka et al., 2002).

CONCLUSIONS

The scientific evidence underscores the significant advantages of blackcurrant cultivation technologies, which have been demonstrated to offer a range of benefits. The increased mechanization of the cultivation process translates to heightened efficiency and reduced labor costs.

The adoption of various soil management technologies, alongside reduced water and pesticide usage through modern applications, contributes to aligning with sustainable agricultural practices and environmental conservation efforts.

Their versatility and resilience, characterized by early fruiting, high yields, and adaptability to varying environmental conditions, contribute to their widespread popularity among growers and expand cultivation options across different regions.

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ANALYSIS OF THE PHENOPHASES OF GROWTH AND FRUITING OF RASPBERRY VARIETIES IN THE CLIMATIC CONDITIONS OF BUCHAREST

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Abstract

The objectives of the research consist in assessing the intensity of the processes that determine the physiological growth of raspberry plants of the Delniwa variety and the Opal variety, which influence the fruiting process and the production of fruits per plant. In the framework of the experiment, the growth and fruiting of the two raspberry varieties under the influence of climatic factors from the experimental location of the raspberry culture established in 2023 within the INMA Bucharest was monitored. Thus, observations were made on the development of the main phenophases of the vegetative organs (foliar) from which the leaves and shoots developed and the fruiting organs (generative / floriferous) from which the flowers developed, hence the harvest. For both varieties, five representative plants of each variety were chosen for monitoring, from which the following parameters were measured every month of the crop`s evolution: the number of shoots, the length and diameter of the stem, the sizes of the fruits and receptacles, as well as the accumulated amounts of fruits specific to each variety analyzed.

Key words*: climatic factors, phenophases, fruits, varieties, raspberries.*

INTRODUCTION

The consumption of small fruits, especially from the genus *Rubus*, which presents an enormous morphological diversity, has increased worldwide, as they are excellent sources of bioactive compounds, especially phenolic compounds, necessary for human nutrition (Fagundes C.M., 2023). If the nutritional components of different raspberry fruits are combined with their high productivity and sensory quality, raspberry varieties are considered to be good quality crops with a high market value. Therefore, by introducing raspberry varieties that possess higher nutritional and antioxidant values, in addition to the standard of high productivity and attractive fruit appearance, it is possible to increase the consumption of healthy fruits (Milivojevic J.M. et al., 2011).

The requirements of raspberries to ecological factors are (Baciu A., 2005; Mihaescu G., 2002; Popescu M. et al., 1982; Sava P., 2017):

• Light: medium requirements;
• Temperature: average optimum: <16-17°C; soil temperature : < 16ºC;
• The relative humidity should be high with annual precipitation of 700-1000 mm (June-August);
- the optimal atmospheric humidity is 70-80% during flowering and 65-70% during the rest of the vegetation period;
- the decrease in relative air humidity below 40% negatively influences physiological processes, and when it reaches values of 20%, assimilation stops.
• Phreatic water: >80 cm;
• Soil: sandy-loamy, fertile, drained, aerated; pH 6.5; rich in nitrogen and protein.

In the paper (Sønsteby et al., 2009) the primocane Polka raspberry variety is presented, similar to the Delniwa variety from the present study, which develops and blooms even at atmospheric temperatures of 30°C, the optimum being at 27°C. But, in the paper (Carew J.G. et al., 2001), the authors present the effects of low temperatures on vegetative growth and flowering of raspberry fruits of the variety

Autumn Bliss. The plants responded significantly to cold treatment and flowering was advanced, indicating a distinct vernalizing effect.

Phenophases at raspberries represent the developmental stages of the raspberry plant, which are influenced by climatic conditions. A study revealed that the average duration of the budding and flowering phases are directly influenced by the temperature values in the months of March-April. A period of 21-65 days passed from the beginning of bud break to the beginning of flowering. A period of 26-69 days passed between the beginning of flowering of raspberry plants and the beginning of fruit ripening (Rusnac C., 2021).

Another study revealed that budding and the beginning of growth mark the start of vegetation, and in the raspberry species it occurred at the end of March, the Benefis variety on the 25th and the Opal variety on the 31st. The raspberry varieties flowered in the second decade of the month May, respectively 12 and 18 (www.cercetarepomicola.ro/).

Phenological evaluations of Primocane raspberry cultivars grown organically in a subtropical region (Fagundes C.M., 2023), respectively a coastal area with a mild climate (Cicala A. et al., 2002) showed two periods of flowering and fruiting, the first in spring/summer and the other in summer/autumn. Phenophases reflect the influence of weather factors on the vegetative and generative development of plants. Each species goes through dozens of phenophases during growing seasons (Rusnac C., 2021; Zejak, D. et al., 2021; Kluza-Wieloch M. et al., 2013; Sava P., 2013). In this sense, the present study analyzes the growth and fruiting phenophases of Opal and Delniwa raspberry varieties, in the climatic conditions of Bucharest.

MATERIALS AND METHODS

The land on which the raspberry culture was established, in March 2023, was represented by an experimental plot of 1000 m^2, located within INMA Bucharest. In order to prepare the land for the planting of the two varieties of cuttings, the land was first scarified, then ground and leveled. The raspberry varieties, scientific name *Rubus idaeus*, planted were cuttings from the Opal variety and cuttings from the Delniwa variety. Both the plant material to be planted and the soil have been ecologically certified.

The slope distance between rows was 3.3 m and between plants 0.5 m for the Opal variety, respectively 0.75 m for the Delniwa variety.

These raspberry varieties were chosen according to the characteristics of the variety, of the fruit, the harvest period, the resistance to the climate of our country, with high rooting power, resistance to frost and pests.

The characteristics of the raspberry varieties used to establish the culture were:

• The floricane variety, the Opal variety (Figure 1), has produced flowers and fruits on stems since spring, fruiting twice a year (replanted), June-July and August-September: the first time in spring on the stems of the previous year and the second time in August-September, on the top of the stems from the current year. This medium-vigorous raspberry variety has bushes with erect stems that bear fruit in the year of formation on about 1/3-1/2 of their length and have a high rooting power. The Opal variety has medium-sized fruits, with small and well-welded drupeoles between them, of a bright red color, rounded shape, intensely aromatic, sweet juicy taste (Popescu M. et al., 1982; Sava P., 2017).

Opal variety raspberry plant, height 40 cm | Ripe Opal variety

Figure 1. Opal cultivar, August 2023

• The primocane variety, the Delniwa variety (Figure 2), in which the emergence of stems, flowering and fruiting took place in the same growing season. The cycle of shoot growth and fruiting occurs in a single year. The Delniwa variety is a variety with fruiting on first-year wood, fruiting from July 15 to November 5-10. It is a Hybrid created by crossing the varieties Polka, Polana and Himbotop, premium variety, with fruits resistant to transport and handling, they do not turn black after harvesting but keep their natural color 2-3 days after harvesting, pink. The Delniwa variety has large fruits, large drupeoles with small seeds inside each welded together, red color, rounded conical shape, intensely aromatic, sweet and sour taste (Popescu M. et al., 1982; Sava P., 2017).

Delniwa variety raspberry plant, height 120 cm   Ripe Delniwa variety

Figure 2. Delniwa cultivar, august 2023

After establishing the raspberry culture to ensure optimal maintenance, biodegradable film was applied for mulching, a support system was installed on trellises and a drip irrigation system. Also, the raspberry stems were straightened during the growing season and the weeds between the plants and between the rows were periodically removed, in order to aerate the plant and to allow growth and fruiting.

Together with the maintenance works, the phenophases of the raspberry culture were monitored: vegetative rest, bud swelling, leafing, flowering and fruit ripening. The growth and fruiting of the two raspberry varieties under the influence of climatic factors from the experimental site of raspberry culture within the INMA Bucharest was followed. Thus, observations were made on the development of the main phenophases of the vegetative organs from which the leaves and shoots developed and the fruiting organs from which the flowers developed, hence the harvest. Raspberry fruits were harvested in stages as they reached maturity.

RESULTS AND DISCUSSIONS

Taking into account the climate changes that evolve every year, it is necessary to carry out a permanent monitoring of the phenological stages of plants, to highlight the process of adaptation of plants, to identify the varieties most suitable for the given area and to intervene with certain technological procedures in the purpose of improving production quality.

As part of the experience, the growth and fruiting of the two varieties of raspberry under the influence of climatic factors from the experimental location of raspberry culture within the INMA Bucharest, for the period March-November 2023, was followed.

The values of the atmospheric parameters monitored with the help of the sensors were: precipitation (P), solar radiation (R), air temperature (Ta), soil temperature (Ts), relative humidity of the air (Ua), relative humidity of the soil (Us), these being shown in the Table 1, and evapotranspiration (ET0) and wind speed are presented in the Figure 3.

Table 1. Atmospheric conditions recorded in the nine months of raspberry vegetation, in the conditions of Bucharest

Month	P, mm	R, [W/m^2]	Ta [°C]	Ts [°C]	Ua [%]	Us [%]
March	16.4	127	8.24	7.4	71.69	41.96
April	63.0	156	10.79	10.5	79.1	42.43
May	28.6	211	16.69	14.8	68.72	28.93
June	14.4	245	21.95	20.1	64.32	14.12
July	13.2	264	25.96	23.8	60.74	12.24
August	0.6	222	26.35	24.5	53.4	10.78
September	0.0	152	22.13	21.9	54.39	9.46
October	0.8	103	15.71	16.4	65.81	8.75
November	53.2	58	8.2	10.8	84.09	11.25

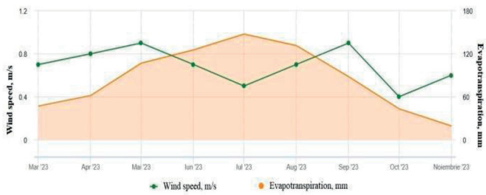

Figure 3. Wind speed and evapotranspiration

The maximum precipitation was recorded in April (63 mm) and November (53.2 mm), and the lowest precipitation was in August (0.6 mm) and October (0.8 mm). No precipitation was recorded in September. The amount of precipitation during March and November was 190.4 mm. The maximum solar radiation was 264 W/m^2 in July.

Regarding the values of air temperature and soil temperature, we note from the Table 1 that in the months of May and October, the values of these parameters are close to those reproduced in the specialized literature (Mihaescu G., 2002), namely optimal temperatures of approximately 16-17^{0}C. Atmospheric humidity does not fall within the optimum of 60-80%, as recommended in the literature (Baciu A., 2005; Mihaescu G., 2002; Popescu M. et al., 1982; Sava P., 2017), in August, September. Also in these months, the soil moisture values were low (below 10%). The maximum wind speed (0.9 m/s) was recorded in May and September and the minimum of 0.4 m/s in October. Evapotranspiration recorded increasing values of over 100 mm from May to August, this being a factor that can easily cause plants to get sick.

During the vegetation and fruiting, from March to November, the plants were monitored and weekly and monthly measurements were recorded over time, in order to see the evolution and development of the cuttings and fruits.

Phenological development stages of raspberry plants

1. ***Vegetative dormancy***

- The period of vegetative rest is usually 6 months, October-March.
- The vegetation period varies from 180 days to 260 days.

2. ***Swelling of buds***

- This stage takes place in March.

3. ***Foliage***

- The first leaves of the Opal variety appeared in April and annual shoots of the Delniwa variety appeared in May.
- For the Opal variety, the leaves were counted in the months of April and May, their number varying from 3 to 5 leaves per plant, then in June and July the side shoots grown on the drajon planted in March were counted, these being between 3 and 9 lateral shoots per plant. In the months of August and September, the annual shoots grown from the root of the dragon plant planted in March were counted, these were 1, 2 or 3 annual shoots depending on each individual plant.
- For the Delniwa variety, the annual shoots grown from the root of the planted dragon fruit were counted, which also bore fruit and their number was from 1 to 4 annual shoots depending on each plant.

4. ***Flowering***

In the experimental varieties, flowering (Figures 4 and 5) occurred:

- for the Opal variety in May on the lateral shoots, then the second flowering took place in mid-August on the annual shoots.
- for the Delniwa variety in June on the annual shoots grown from the root of the planted cutting.

Figure 4. Foliage and flowering in the Opal variety

Figure 5. Foliage and flowering in the Delniwa variety

5. ***Fruit ripening***

- In the Opal variety, the first ripening of the fruits took place in the June-July period and the second ripening in the September-October period (Figure 6), depending on the weather conditions.
- In the Delniwa variety, the fruits ripened from the end of July until November (Figure 7).

Figure 6. The first ripening (left)
and the second ripening (right) of the Opal variety

Figure 7. Fruit ripening in the Delniwa variety

The fruits of the two varieties (Figure 8) present the following characteristics:

• The Opal variety has medium-sized fruits, with small and well-welded drupeoles between them, of a bright red color, rounded shape, intensely aromatic, sweet juicy taste, weighing between 2.5 and 1.2 grams, receptacles with heights between between 0.6-0.9 cm (Figure 9).

• The Delniwa variety has large fruits, large drupeoles with small seeds inside each welded together, red color, rounded conical shape, intensely aromatic, sweet and sour taste, weighing between 6.1 and 4.3 grams, receptacles with heights between 0.4-2.0 cm (Figure 9).


Opal variety


Delniwa variety

Figure 8. Raspberry fruit sizes


Opal variety


Delniwa variety

Figure 9. Dimensions of the receptacles of raspberry

Figure 9 shows the shape and dimensions of the receptacles of the two raspberry varieties.

In both varieties, daily observations and monthly measurements of stem length and diameter were made (Tables 2 and 3), as well as the number of fruits and their quantities accumulated monthly on certain plants, depending on the variety.

The average measurements for the height of the Opal variety plants varied from 35.5 cm for plant 2 to 65.7 cm for plant 4, and the stem diameter varied from 4.4 mm for plant 2 to 8.1 mm for plant 1.

The average measurements for the height of the Delniwa variety plants varied from 34.6 cm for plant 5 to 64.9 cm for plant 3, and the stem diameter varied from 8.6 mm for plant 2 to 12.2 mm for plant 2.

Table 2. The dimensions of the plants of the Opal variety

	Plant height (cm)				
Month	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**
April	36.00	30.00	38.00	35.00	35.00
May	45.50	31.50	39.50	40.00	37.50
June	49.00	32.50	45.10	46.00	44.00
July	55.00	34.00	51.00	49.00	51.00
August	71.00	37.00	61.00	91.00	96.00
September	91.00	48.00	116.00	133.00	121.00
	Plant stem diameter (mm)				
April	5.29	4.41	6.73	5.21	5.86
May	5.68	4.66	7.14	5.37	4.83
June	6.59	5.75	7.73	6.09	5.11
July	9.22	3.03	5.47	6.26	6.99
August	10.22	3.58	7.75	9.05	11.54
September	11.88	5.21	8.57	12.23	12.4

Table 3. The dimensions of the plants of the Opal variety

	Plant height (cm)				
Month	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**
April	20.00	22.00	24.50	13.50	19.50
May	26.00	26.50	28.00	15.00	24.10
June	32.00	40.00	37.00	39.00	31.00
July	53.00	76.00	86.00	45.00	41.00
August	52.00	97.00	102.00	81.00	37.00
September	55.00	111.00	112.00	130.00	55.00
	Plant stem diameter (mm)				
April	6.00	8.23	6.12	7.20	6.10
May	6.94	8.84	6.86	7.70	6.98
June	7.78	9.37	10.55	8.70	7.39
July	9.23	12.19	11.85	9.37	9.43
August	10.16	16.27	15.81	11.04	10.22
September	11.23	18.42	16.41	12.72	12.04

The figures show the monthly production from the first year correlated with the number of fruits for each of five randomly chosen plants from the establishment of the culture for the two raspberry varieties.

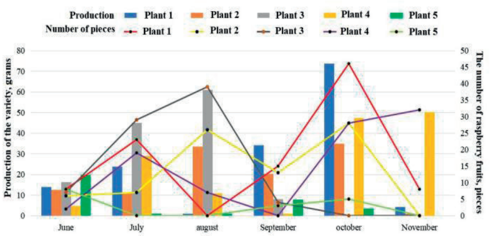

Figure 10. Raspberry crop production, Opal variety

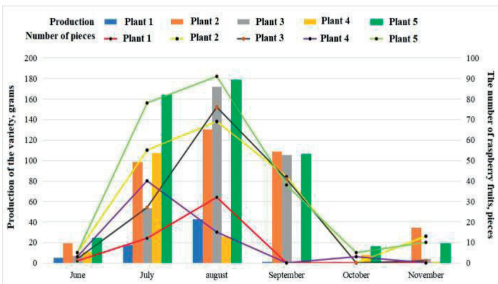

Figure 11. Raspberry crop production, Delniwa variety

From the analysis of Figure 10, it can be seen for the Opal variety that at plant 1, 151.1 g was obtained per 100 pieces, at plant 2, 113.5 was obtained at 80 pieces, at plant 3, 130.6 g was obtained at 79 pieces, at plant 4, 143.5 g was obtained from 88 pieces and at plant 5, 33.4 g was obtained from 16 pieces of fruit. The average variations of the weight of a fruit were 1.42-2.08 g.
From the analysis of Figure 11, it can be seen for the Delniwa variety that at plant 1, 68.5 g was obtained from 45 pieces, at plant 2, 391.5 was obtained from 184 pieces, at plant 3, 342.4 g was obtained from 147 pieces, at plant 4, 154.2 g was obtained from 61 pieces and at plant 5, 508.4 g was obtained from 227 pieces of fruit. The average variations of the weight of a fruit were 1.52-2.52 g.

CONCLUSIONS

From the correlation of the atmospheric conditions recorded in the nine months of vegetation of the year 2023, in the conditions of Bucharest, with the productions obtained in the two raspberry varieties studied, the following was found:
- the Opal variety produces small fruits, a plant of this variety can produce between 16 pieces with a total weight of 33.4 g per plant and up to 100 pieces with a total weight of 151.1 g per plant; the first ripening of the fruits in June-July on the lateral shoots of the cuttings planted in March and the second ripening in September - October on the annual shoots grown from the root of the cutting planted in spring; the dimensional characteristics of the plants: (Table 2) the smallest growth in height of 18 cm in plant 2 and the largest growth of 98 cm in plant 4; stem diameter of 0.8 mm in plant 2 and 7.02 mm in plant 4.
- the Delniwa variety produces large fruits, a plant of this variety can produce between 45 pieces with a total weight of 68.5 g per plant and up to 227 pieces with a total weight of 508.4 g per plant; ripening was later and longer from July to November, with maximum amounts of ripening between July and September; the dimensional characteristics of the plants (Table 3) the smallest growth in height of 35.0 cm in plants 1 and 5, and the largest growth of 116.5 cm in plant 4; stem diameter of 5.2 mm in plant 1 and 10.29 mm in plant 3.
The analysis of the growth and fruiting phenophases can be an indication of the intensity of the physiological processes that determine plant growth and that influence the fruiting process and the production of fruits per plant.
Knowing and establishing the phenophase of the plant is important for farmers in the context in which it allows certain works, operations to be carried out at the optimal time, for example such as combating diseases, pests, cutting.
In general, the good growth of raspberries for both varieties indicates that the production of Opal and Delniwa raspberry varieties can be implemented in Bucharest (Baneasa area), which can provide a continuous and long-term supply of fresh raspberries on the market, as well as fruits good quality.

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EVALUATION OF SOME ESSENTIAL ELEMENTS IN WALNUT KERNEL

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Abstract

The paper presents the results in determining essential element content in walnut kernel marketed in agri-food markets in Timisoara (Romania) and in estimating the supply of minerals of this food drug. Results of mineral elements analysis through flame atomic absorption spectrometry show that walnut kernel analyzed contains increased amounts (mg/100g) dry matter of K (338-457), Mg (163-270) and Ca (62.60-119) and significant amounts of Mn (2.56-10.40), Fe (l.19-5.58), Zn (1.23-3.58), Na (0.75-6.45), Cu (0.58-3.14), Cr (0.08-0.60), unevenly distributed according to the origin of the kernel and the nature of the analyzed element. The results obtained when evaluating the mineral intake, show that a consumption of 25 g of walnut kernel contributes differently to ensuring the daily mineral needs: is not of interest from the point of view of the contribution of Na, Ca and K, but can cover significant percentages of the required Zn, Fe and Mg and high Cr, Cu and Mn. Given the results of our experiment, we can say that the analyzed walnut kernel can be considered as a good additional source, especially of Cr, Mn, Cu, Mg and less as the source of Zn and Fe.

Key words: *walnut kernel, essential element, mineral intake.*

INTRODUCTION

Walnut, the fruit of the Juglas tree directed L., is the most valuable of all hard-shelled fruits (Ros, 2010). Walnuts contain a lot of nutrients, on average: 60-70% lipids (especially mono and polyunsaturated fats), 12-24% protein, 12-18% carbohydrates, 6.7% dietary fiber and 1.7-2.0% minerals (Simsek, 2016; Cosmulescu, 2009; Leahu et al., 2013). Walnut kernel, the edible part of walnut, is one of the most appreciated fruits and it is an excellent source of essential nutrients such as: essential minerals, carbohydrates, phenolic compounds, vitamins and omega-3 and omega-6 polyunsaturated fatty acids etc. (Cindrić et al., 2018; Şen and Karadeniz, 2015; Trandafir et al., 2016); Yilmaz and Akça, 2017; Al-Snafi, 2018; Momchilova et al., 2016). Walnut kernel are a rich source of α-linolenic acid, phytosterols, nonsodium minerals, γ-tocopherol, melatonin and polyphenols (Ros, 2018). Walnut kernels also contain important vitamin structures such as riboflavin, niacin, thiamine, pantothenic acid, vitamin B_6 and folates (Şen and Karadeniz, 2015).

Regarding the composition of minerals, walnut is considered a good source of minerals. Potassium (K), phosporous (P), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), mangane Mn, iron (Fe), selenium (Se), chromium (Cr), etc. (Simsek, 2016; Cindrić et al., 2018; Trandafir et al., 2016, Yilmaz et Akça, 2017; Chatrabnous et al., 2018; Polat et al., 2015; Ozyigit et al., 2018; Rada et al., 2018; Cosmulescu et al., 2010; Zhai et al., 2014), essential for the normal functioning of the human body. Walnuts are low in Na, but rich in K, Mg, and Ca. These three minerals are involved in many aspects of cellular metabolism and other biological processes, including insulin sensitivity, blood pressure regulation and vascular reactivity (Ros, 2018). Microelements, such as Fe, Cu, Zn, Mn, and Se are essential components of biological structures and have an important effect on and play a key role in a variety of the processes necessary for life throughout mediate vital biochemical reactions (Al-Fartusie and Mohssan, 2017).

The special quality and richness of bioactive nutrients and phytochemicals that are part of the walnut kernel also confers numerous therapeutic qualities: reduce risk of developing cancer, cardiovascular risk decreases, help to reduce body weight, improve endocrine parameters in polycystic ovary syndrome, help to control diabetes, improve thinking ability etc. (Şen and Karadeniz, 2015; Ros, 2018; Fatima et al., 2018; Kalkişim et al., 2014)**.**

The fact that in the walnut kernel, the edible part of the walnut fruit (*Juglans regia L.*), are concentrating increased amounts of essential mineral elements is supported by numerous studies in the field published in the last ten years.

In the analysis of walnut kernels from some walnut cultivars in Kirşehir province (Turkey), Özcan et al. determined important concentrations of essential mineral elements: 11.99-15.11 mg/kg B, 2462-2758 mg/kg Ca, 1.695-3.323 mg/kg Cr, 5.676-5.944 mg/kg Cu, 17.88-21.82 mg/kg Fe, 5380-3478 mg/kg K, 4163-5488mg/kg Mg, 17.59-22.20 mg/kg, Mn, 1.671 mg/kg Mo, 617-833 mg/kg Na, 1.651-1.915 mg/kg Ni, 2226-2604 mg/kg P and 0.623-17.981 mg/kg - Zn (Özcan et al., 2010).

Turkish walnuts were also investigated for contamination with heavy metals. Kalkişim et al., determined the levels of essential and non-essential elements in walnut powder (*Juglans regia* L.) collected near and far from the Gumushane highway area. The average concentrations of trace elements Al, Cd, Cr, Cu, Fe, Ni, Mn, Zn, Ba, Na and Sr in the walnut samples collected near the motorway area were (μg/g): 10.39, 0.055, 0.503, 15.82, 32.54, 1.16, 19.96, 21.48, 3.90, 116.12, respectively, 3.22 (Kalkişim et al., 2014). Macro-elements: Ca, K, and Mg were determined in much higher concentrations: 0.72, 3.40, respectively, 1.15 mg/g (Kalkişim et al., 2014). Comparing the values obtained with the average data obtained for the control samples, the study authors observed that the levels of Al, Cd, Cr, Cu, Ca, K, Na and Sr are higher in the walnut samples collected near the highway area. Kafaoğlu et al. reported increased amounts of macroelements and significant amounts of trace elements: Mg (1034.96 mg/kg), Ca (793 mg/kg), B (15.5 mg/kg), Co (29.11 μg/kg), Cu (11.73 mg/kg) Fe (22.08 mg/kg), Mn (24.23 mg/kg), Ni (718.66 μg/kg) and Zn (23.25 mg/kg) (Kafaoğlu et al. 2016). The fact that in the composition of the walnut kernel from different genotypes and varieties of walnut (*Juglans regia* L.) from Turkey are concentrated increased amounts of K, Mg and Ca as well as significant amounts of Na, Fe, Mn, Zn, Cu is to be demonstrated and the works elaborated by Polat et al. (2015), Simsek (2016) and Yilmaz et al. (2017). The authors of these studies showed that the walnut kernel samples have the following mineral profile (mg/100 g): K (408.37-569.48), Ca (94.79-267.85, Mg (241-426), Cu (0.72-1.43), Zn (1.93-3.47) (Polak et al., 2015); K (534.3-778.6), P (346.0-584.8), Ca (100.9-233.9) and Mg (117.8-181.4), Na (8.67–19.29), Fe (3.13–5.37), Mn (2.02-4.50), Zn (1.44-3.63) and Cu (0.77-2.44) (Simsek, 2016); P (230.65-344.40), K (163.92-308.86), Na (7.94-22.53), Cu (2.25-364), Fe (2.21-4.32), Zn (1.97-5.48), Mn (0.91-439) (Yilmaz and Akça, 2017).

The Persian walnut kernel was also analyzed by Aryapak and Ziarati, which when evaluating the traditional walnut kernel in Teheran and Karaj (Iran) determined high concentrations (mg/100 g dry weight) of: Na (58.42-66.54), K (546.46-617.36), Ca (235.19-276.46), Mg (580.11-692.49); Fe (444.02-559.52), Cu (281.20-322.73), Se (0.003) and Zn (133.92-142.0) (Aryapak and Ziarati, 2014). These nuts, are distinguished by high contents of Fe, Cu, Zn, and Na.

Important data regarding the mineral profile are provided by Ebrhimi et al. which in a study on the importance of Persian walnuts (*Juglans egia* L.) in human health, shows that the Persian walnut kernel is a food rich in essential minerals (mg/100 g): 358, 414, 372.69, and 340 K; 362 P; 153, 316, 140, and 115 Mg; 125, 61, 120, and 72 Ca; 3.8, 4.6, and 3.2 Fe; 4.4, 6.4, 7.4, and 11.5 Na; 3.8, 2.12, and 2.2 Cu (Ebrhimi et al., 2018).

Interest in knowing the concentrations of mineral elements in walnut kernels from cultivated walnut species or from various domestic markets have also been reported in papers published by researchers in China. Analyzing the mineral concentration of walnut kernel from 17 types of walnut (*Juglans regia*

L.) from China, Zhai et al. determined increased concentrations (mg/100 g) of Mg (401.47-749.27) and Ca (253.90-504.07), as well as significant amounts (mg/100 g) of Fe (4.44-10.45), Mn (2.73-17.12), Zn (6.84-13.32) and Cu (1.37-5.21) (Zhai et al., 2014). Proceeding to determine some elements that have essentiality or toxicity for the body from the walnut kernel marketed in Chinese markets, Ni et al. and Yin et al., found that this food contains different amounts (mg/kg) of essential and potentially toxic elements: 0.24-0.35 (Cr); 34.4-57.9 (Mn); 34.3-44.5 (Fe); 0.32-0.67 (Mo); 9.30-24.7 (Cu); 14.8-27.7 (Zn); 0.035-0.072 (Se); 8.43-14.2 (Sr), 0.99-2.22 (Al); 0.0032-0.013 (As), 0.012- 0.022 (Cd); 0.0051-0.012 (Pb) (Ni et al., 2016), respectively: 10 µg/g, Mn; 0.02 µg/g, Co; 8.8 µg/g, Cu; 20 µg/g, Zn; 0.01 µg/g, Li; 0.12 µg/g, As; 2.9 µg/g, Ru; 6.4 µg/g, Sr; 0.45 µg/g Ba; 0.11 µg/g Mo; 0.02 µg/g Cd (Yin et al., 2015). Finally, the authors of these studies consider that the analyzed walnut kernel samples show appreciable concentrations of essential elements and a normal consumption of walnut kernels does not present a risk of contamination with toxic metals.

Arpadjan et al. determined the distribution of essential and toxic elements in 12 types of nuts grown in two locations in Bulgaria: an area contaminated with lead metallurgy and an area without pollution. Average values obtained (mg/kg): 0.013-0.045, Cd; 0.05-0.38, Co; 0.032-0.076, Cr; 15.4-20, Cu; 28-33, Fe; 31-36, Mn; 0.64-1.62, Ni; 0.08-0.20, Pb; 0.006-0.01, Sb; 0.034-0.047, Se; 28-36, Zn - in the polluted area and 0.08-0.12, Co; 0.030-0.038, Cr; 15.3-17.4, Cu; 23-38, Fe; 28-35, Mn; 0.40-0.96, Ni; 0.034-0.047, Se; 28-34, Zn; 0.005-0.009, Cd; <0.01-0.04 Pb; <2, Sb - in the unpolluted area, shows a general tendency to increase the Cd, Co, Cr, Ni and Pb content in the contaminated area (Arpadjan et al. 2013). Under these conditions, the average intakes of Co, Cr, Cu, Fe, Mn, Ni, Se and Zn corresponding to a consumption of 50 g of walnut kernels in the polluted area were below the toxicological reference values. Also, the maximum exposure of nuts to Cd, Pb and Sb represented only 10.5%, 4.7% and 0.1% of the maximum tolerable weekly intakes. Therefore, walnuts can be grown even in a polluted area, without losing their nutritional qualities. Analyzing the distribution of Cd, Cu, Fe, Mn, Pb, Zn in six varieties of walnuts (*Juglans regia* L.) grown in Bulgaria, Sv. Momchilova et al. (2016) found that the concentration (mg/kg) of the analyzed elements: 0.0235 - 0.0298 (Cd); 15.7-17.2 (Cu); 24.2-32.8 (Fe); 32.0-39.5 (Mn); 0.095-120 (Pb) and 26.8-34.2 (Zn), is conditioned by the walnut variety (Momchilova et al., 2016).

Cindrić et al. also concluded that walnut is an important element which when analyzing the core of some mineral elements from walnut kernel products from France and Canada determined high quantities (mean values in mg/kg) of Ca (1062), K (2772), Mg (1426) and significant amounts of Na (42.5), Mn (31.7), Fe (27.4), Zn (24.0) and Cu (8.38); microelements with toxic properties: As, Cd, Pb, were determined in concentrations (mean values, mg/kg) below the maximum allowed limit: 0.027, 0.0039, respectively 0.331 (Cindrić et al., 2018).

Proceeding to evaluate the bioaccessibility of some essential macro and microelements: Ca, Mg, Fe and Zn from walnut (*Juglans regia* L.), Suliburska and Krejpcio showed that the walnut kernel from the local market from Poznan (Polska) contains increased amounts of Mg (140.1 mg/100 g) and Ca (73.1 mg/100 g) and notable amounts of Fe (2.1 mg /100 g) and Zn (1.8 mg/100 g) (Suliburska and Krejpcio, 2014).

When analyzing some mineral nutrients and heavy metals from walnut kernel samples collected from four areas of natural walnut-fruit forests in Osh Region (Kyrgyzstan), Ozyigit et al. determined high amounts (mg/100 g) of K (311.6-397.1), Ca (138.5-152.1), Mg (158.6-220.1), as well as important quantities of: Mn (3.045-4.562), Fe (3.356-4.892), Zn (2.320-3.463), Cu (1.251-2.067) and Na (0.878-1.700) (Ozyigit et al., 2018). The experimental results obtained allowed the evaluation of the mineral intake in the daily diet, for a certain consumption of walnut kernels. Under the conditions described by the study authors, a daily consumption of 100 g of walnut kernel covers a large part of the recommended mineral requirement: Ca (15%), Cu (173%), Fe (52.25%), K (7.6%), Mg (45.40%), Mn (135.7%), Na (0.09%), Zn (26.8%) for adults and Ca (15%), Cu (173%), Fe (23.22%), K

(7.6%), Mg (59.5%), Mn (211.1%), Na (0.09%), Zn (36.9%) for premenopausal women).

Concerns for knowing the mineral profile of the core are also recorded in the works of researchers in Romania, which show that the Romanian walnut kernel is a rich source of essential mineral elements, and the consumption of walnut kernels contributes to a well-balanced diet. Analyzing the mineral composition of some walnut kernels obtained from nine local walnut varieties, Cosmulescu et al. determined identified high contents (mg/100 g) of essential elements, unevenly distributed, depending on the variety of walnut and the analyzed element: 0.134-2.387, Na; 357.1-499.6, K; 37.0-90.84, Ca; 189.2-234.2, Mg; 3,815-5,927, Fe; 3,134-18.37, Mn; 1.41-3.223, Cu; 0.002-0.005, Se; 0.102-0.525, Al; 0.255-0.692, Cr; 0.160-0.537, Sr .; 0.001, V; 0.356-2.607, Rb; 1,948-3,613, Zn (Cosmulescu et al., 2009). In another study, Cosmulescu et al., determined the distribution of Na, K, Ca, Mg, Fe, Mn, Cu, Se, Al, Cr, Zn, Sr and Rb, from walnut kernels from three varieties of Romanian walnut. The concentration of these elements (mg/100 g) in the analyzed walnut kernel varies in relatively wide limits between: 387.25-444.35, K; 264.7-272.3, Mg; 62.78-72.91, Ca; 10.45-18.06, Mn; 5.44-5.90, Fe; 3.19-4.10, Zn; 2.93-3.47, Cu; 0.17-0.33, Al; 0.59-0.84, Cr; 0.39-0.55, Sr.; 0.13-0.56, Rb. (Cosmulescu et al., 2010). Analyzing some nutritional and functional components of the walnut kernel and pellicle across twelve genotypes of *Juglans regia* L. (six with a red pellicle and six with a light yellow pellicle), Trandafir et al. reported that walnuts are a good dietary source of total phenolics with high antioxidant potential, minerals (Ca, Mg and K) and essential elements (Fe, Mn, Cu and Zn), most of which are concentrated in the walnut pellicle (Trandafir et al., 2016). The concentration of essential elements (mean values/dry matter) analyzed presents the following values: 74.82 mg/ 100 g, Ca; 228.45 mg/100 g, Mg; 354.11 mg/100 g, K; 0.62 mg/100 g, Na; 3.89 mg/100g, Fe; 11.16, Mn; 2.14, mg / 100 g, Cu; 0.50 mg/100 g, Cr; 2.89 mg/100 g, Zn - in walnut kernel with red pellicle and 60.82 mg/100 g, Ca; 202.95 mg/100 g, Mg; 764.99 mg/100 g, K; 0.97 mg/100 g, Na; 3.50 mg/100 g, Fe; 6.83 mg 100 g, Mn; 2.03 mg/100 g, Cu; 0.68 mg/100 g, Cr; 2.96 mg/100 g, Zn - in walnut kernel with a light yellow pellicle (Trandafir et al., 2016). More recent data on the concentration of some essential microelements in the Romanian walnut kernel are presented by Rada et al. (Rada et al., 2018). The study authors reveal that the analyzed walnut kernel contains significant amounts (mg/kg) of Mn (18.59-51.65), Fe (20.5-29.1), Zn (16.06-26.15, Cu (9.64-10.07) and reduced amounts of Cr (0.78-1.08). These values allowed the evaluation of the mineral intake of the walnut kernel analyzed in the diet of people aged between 19 and 50 years. The authors of the study show that a daily consumption of 20 grams of walnut kernels covers a certain percentage of the daily mineral requirement: Cr (51.43%), Mn (34.15%), Cu (22.31%), Fe (6.13%), Zn (3.58%) - for man and Cr (72.00%), Mn (43.63%), Cu (22.31), Zn (4.93%), Fe (2.72%), - for women.

From the above values it can be stated that the walnut kernel contains increased quantities of essential mineral elements, distributed differently depending on a series of factors: variety, soil and climatic conditions and culture, nature of the element, etc. To evaluate the nutritional intake of walnut, it is very important to determine the concentrations of these essential elements. The purpose of this study is to determine the concentrations of some essential elements: Na, K, Ca, Mg, Fe, Mn, Zn, Cu and Cr from walnut kernel samples sold in agri-food markets in Timisoara (Romania) and evaluating the mineral intake of this precious food.

MATERIALS AND METHODS

Samples

To carry out this study, five samples of walnut kernels were taken from agri-food markets in Timisoara (Romania). Until the time of analysis, these walnut kernel samples, from the 2018 harvest, were kept cold (about 4-5ºC) in plastic bags.

Instrumentation

- Nabertherm thermoregulatory calcination furnace model 6/11, used to burn walnut kernels;

- Varian 280 FS atomic flame absorption spectrophotometer, used to measure the absorbances of mineral elements in standard solutions and in the solution of problems obtained after mineralization.

Chemicals and Reagents

- Merck nitric acid, 65% (ρ = 1.39 g/cm^3), required for the preparation of the 0.5 N nitric acid solution;
- Multi - element standard solution (1 g/L) Merck - Germany, used in the preparation of working standards for Na, K, Ca, Mg, Fe. Mn, Zn, Cu and Cr, in concentrations covering the concentration range of the elements present in the analyzed samples;
- Double-distilled water.

Method

The determination of the concentrations of the mineral elements in the walnut kernel samples was performed by the atomic absorption method in the air-acetylene flame according to the procedure described by Rada et al. and Yerlikaya et al. (Rada et al., 2018; Yerlikaya et al., 2012).

Basically, 1±0.0002 g of walnut kernel, previously ground and homogenized were calcined at 550°C in two batches of 4 hours each. To prevent loss of the sample during calcination, before calcination the sample was treated with 2 mL 65% HNO_3, after which it was evaporated on an electric hob to dryness. After cooling, the ash obtained was treated with 20 ml of 0.5 N HNO_3 solution, after which it was evaporated (on an electric hob) until almost sec. This operation was repeated twice, after which it was brought quantitatively with small portions of 0.5 N nitric acid and distilled water at 50 ml. The determination of the concentrations of the elements in the clear solution brought to the level of 50 ml was performed with the help of the Varian 280 FS Spectrometer, in the air-acetylene flame. Working parameters of the atomic absorption spectrometer: wavelength, air flow and acetylene, etc. were recommended by the device supplier. Simultaneously with the measurement of the analyzed samples and in the same working conditions, the calibration solutions were measured. All the mineral composition analyses were performed in triplicate.

Statistical analysis

The average values of the concentrations of the mineral elements were calculated using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA), and then they were statistically tested in order to find significant differences by the T-Test: Two-Sample Assuming Unequal Variances was used for p = 0.05.

RESULTS AND DISCUSSIONS

The results obtained in determining the essential mineral elements from the walnut kernel samples studied are presented in Table 1.

Table 1. The concentration of some essential element in walnut kernel

Specification	Mineral element, mg/100 g*								
	Na	K	Ca	Mg	Mn	Fe	Zn	Cu	Cr
Source 1	4.32± 0.16^a	401± 15.89^a	112± 10.03^a	226± 14.17^a	3.93± 0.36^a	2.45± 0.23^a	1.97± 0.22^a	1.02± 0.21^a	0.19± 0.09^a
Source 2	1.22± 0.10^b	412± 15.90^b	80.1± 8.67^b	270± 15.58^b	9.52± 0.51^b	5.58± 0.39b	3.49± 0.27^b	3.14± 0.26^b	0.49± 0.14^b
Source 3	0.75± 0.08^b	376± 14.20^c	75.2± 7.68^b	221± 8.27^a	10.4± 08.4^b	3.71± 0.31^c	1.48± 0.20^a	2.10± 0.20^c	0.60± 0.15^b
Source 4	6.45± 0.47^a	338± 10.20^d	62.6± 6.16^b	163± 10.98^c	2.56± 0.24^a	1.19± 0.21^a	1.23± 0.31^a	0.58± 0.11^a	0.16± 0.05^a
Source 5	1.93± 0.14^b	457± 18.55^b	119± 6.68^a	176± 10.03^c	3.05± 0.38^a	4.22± 0.26^e	3.58± 0.28^b	1.86± 0.18^a	0.08± 0.02^a
Mean value	***2.93***	***397***	***89.8***	***211***	***5.89***	***3.43***	***2.35***	***1.74***	***0.30***

*a-e: Values in the same column not sharing a common superscript differ significantly (P< 0.05)

As can be seen from this table, the walnut kernel contains increased amounts of K, Mg, Ca as well as significant amounts of Na, Fe, Mn, Zn, Cu and Cr unevenly distributed, depending on the origin of the walnut kernel and the nature of the element analyzed. This non-uniformity, even within each analyzed element, can be explained by the origin of the analyzed walnut kernel. The best represented among the analyzed elements are the macro elements (average values): K (397 mg/100 g), Mg (211 mg/100 g) and Ca (89.8 mg/100 g), which represent a mass weight of 97.59% of the total analyzed elements. The other ingredients were determined in much smaller amounts, as follows: 2.93 mg/100 g Na, 5.89 mg/100 g Mn, 3.43 mg/100 g Fe, 2.35 mg/100 g Zn, 1.74 mg/100 g Cu and 0.30 mg/100 g Cr, but high enough given the quantities needed for the normal functioning of the human body.

Considering the average concentrations of the analyzed mineral elements, it is stated that their distribution in the walnut kernel samples studied (mg/100 g) shows the following decreasing trend (in mg/100 g): K (397) > Mg (211) > Ca (89.8) > Mn (5.89) > Fe (3.43) > Zn (2.35) > Cu (1.74) > Cr (0.30).

Regarding the total concentrations of the mineral elements determined in the walnut kernel samples from the five sources: 752.88 mg/100 g - from S1, 785.54 mg/100 g - from S2, 691.24 mg/100 g - from S3, 575.77 mg/100 g - from S4 and 766.72 mg/100 g - from S5, the statistical analysis performed for p = 0.05 ensures the existence of significant differences in favor of the S2 source, closely followed by S5 and S1. Sources S3 and S4 are significantly different from those mentioned above. As such, source S2 is the richest source of minerals, and source S4 is the poorest source of minerals.

Potassium is the main cation in intracellular fluid with important role in acid-base balance, regulation of osmotic pressure, conduction of nerve impulses, muscle contraction particularly the cardiac muscle, cell membrane function and Na+/K+ - ATPase (Cosmulescu et al., 2010; Soetan et al., 2013). This macro element is the best represented of all the analyzed elements, the concentration limits being between 338-457 mg/100g. Statistically, the highest potassium concentrations were determined in walnut kernels from sources S5 and S2, values without statistically significant differences. Lower values were identified in the sample from source S1, respectively from sources S3 and S4 (these two statistically significant differences). These values are comparable to those reported in literature: 347.8757-547.6201 mg/100 g (Özcan et al., 2010), 349, 358, 372.69, 414 mg/100 g (Ebrhimi et al., 2017), 387.23-444.35 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 354.11 mg/100 g - kernel with red pellicle (Trandafir et al., 2016), but lower than these: 408.37-569.4 mg/100 g (Polat et al., 2015), 534.3-778.6 mg/100 g (Simek, 2016), 546.46-617.36 mg/100 g (Aryapak and Ziarati, 2014), 746.99 mg/100 g - kernel with yellow pellicle (Ros, 2018). The higher values were reported by Yilmaz and Akça, 2017 (63.92-308.86 mg/100 g), Cindrić et al., 2014 (200.6-322.1 mg/100 g).

Magnesium, as an essential macroelement is a remarkable cofactor, necessary for ATP synthesis, glycolysis, oxidative phosphorylation, protein synthesis, muscle contraction, neuromuscular conduction, bone structure, blood sugar metabolism, membrane stabilization, and DNA transcription (Ozyigit et al., 2018). In addition, it is involved in immune system processes (Ozyigit et al., 2018). This element was determined in lower concentrations than potassium but higher than calcium and much higher than the other elements. The highest concentration of Mg was identified in walnut kernel from source S2, followed by walnut kernel from sources S1 and S3 (no statistically significant differences) and S4 and S5 (no statistically significant differences). The limits of Mg concentrations determined in the analyzed walnut kernel (163-270 mg/100 g), are comparable to those obtained by Ozyigit et al., 2018 (158.6-220.1 mg/100 g), Cosmulescu et al., 2009; Cosmulescu et al. 2010 (189.2-234.2, and 264.7-272.3 mg/100 g), Trandafir et al., 2016 (202.95, 228.45 mg/100 g). Magnesium concentrations in the analyzed walnut kernel samples show lower values than those obtained by Özcan et al.(2010): 416.36-548.81 mg/100g, Polat et al. (2015): 241-426 mg/100 g, Aryapak et al. (2014): 580.11-692.49 mg/100 g, Zhai et al. (2014): 401.47-749.27 mg/100 g and higher than those obtained by Kalkışım et al. (2014): 115 mg/100 g, Kafaloglu (2016): 103,496 mg/100 g, Simsek (2016): 117.8-181.4 mg/100 g, Ebrhimi et al. (2017): 115 and 140 mg/100 g.

Calcium, an essential macro element, is an important constituent of bones and teeth, helps regulate nerve and muscle function and is important for blood clotting, blood pressure regulation, immune system health (Soetan et al., 2010). The walnut kernel analyzed contains high amounts of Ca, but lower than K and Mg and less than the rest of the elements analyzed. The concentration limits determined experimentally, between 62.6-119 mg/100 g, are comparable to the values reported: 79.3 mg/100 g (Kafaloglu, 2017), 72 mg/100 g (Kalkışım et al., 2014), 86.6–143.5 mg/100 g (Cindrić et al., 2018), 73.1 mg/100 g (Suliburska and Krejpcio, 2014), 37.0-90.84 and 62.78-72.91 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 74.82 mg/ 100 g - kernel with yellow pellicle (Trandafir et al., 2016), but smaller than those determined by 246.2315-275.7883 mg/100g (Özcan et al. 2010), Polat: 194.79-267.85 mg/100 g (Polat, 2015), Simsek: 100.9-233.9 mg/100 g (Simsek, 2016), 235.19-276.46 mg/100 g (Aryapak et al., 2014), 253.90-504.07 mg/100 g (Zhai, 2014), 138.5-152.1 mg/100 g (Ozyigit et al. 2018).

The highest concentrations of calcium, with no statistically significant differences, were determined in samples from S1 and S5 sources. Lower calcium concentrations with no statistically significant differences were identified in samples from S2, S3 and S4 sources.

Sodium is a macro element that helps regulate the osmotic balance of all solutions in the body and adjusts the volume of intra/intercellular solutions; along with K, establishes the electrochemical potential (membrane potential) (Ozyigit et al., 2018). This macro element that helps regulate blood volume, thus affecting blood pressure was determined in very low concentrations compared to the first three macro elements, the concentration limits being between 0.75-6.45 mg/100 g core. The highest Na concentrations, with no statistically significant differences, were determined in sources S4 and S1. The rest of the samples show lower Na concentrations, but without statistically significant differences. Experimentally determined Na concentrations are comparable to those reported: 4.4 and 6.0 mg/100 g (Ebrhimi et al., 2017), 1.87-19.43 mg/100 g (Cindrić, 2018), 0.878-1.70 mg/100 g (Ozyigit et al. 2018), 0.134-2.387 and 0.23-1.38 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.97 mg/100 g - kernel with yellow pellicle (Trandafir et al., 2016) and lower compared to those reported: 61.7713-833.433 mg/100 g (Özcan et al., 2010), 11,612 mg/100 g (Kalkışım et al., 2014), 8.67-19.29 mg/100 g (Simsek, 2016), 7.94-22.53 mg/100 g (Yilmaz and Akça, 2017), 58.42-66.54 mg/100 g (Aryapak and Ziarati, 2014).

Manganese is an essential microelement that helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation (Al-Fartusie and Mohssan, 2017; Ozyigit et al., 2018). Manganese is the best represented among the analyzed microelements, its concentration presenting values between 2.56-10.04 mg/100g, values comparable to those reported: 2.02-4.05 mg/100 g (Simsek, 2016), 0.91-4.39 mg/100 g (Yilmaz and Akça, 2017), 3.44-5.79 mg/100 g (Ni et al., 2015), 2.8-3.6 mg/100 g (Arpadjan et al., 2013), 1.37-4.6 mg/100 g (Cindrić, 2018), 3.045-4.462 mg/100 g (Ozyigit et al., 2018), 3.134-18.37 mg/100 g (Cosmulescu et al., 2009), 1.859-5.165 mg/100 g (Rada et al., 2018).

The highest concentrations of Mn were determined in walnut kernels from sources S3 and S2 (no statistically significant differences). At lower concentrations, but without statistically significant differences was determined in the walnut kernel from sources S1, S5 and S4.

Iron is an essential component of myoglobin, a protein that provides oxygen to muscles and is necessary for the growth, development, normal functioning of cells and the synthesis of hormones and connective tissue (Al-Fartusie and Mohssan, 2017). The concentrations of this essential microelement in the analyzed walnut kernel show values between l.19-5.58 mg/ 100 g. Statistically, the highest concentration in Fe was determined in the walnut kernel from source S2. Lower concentrations, statistically significantly different, were identified in samples from sources S5, S3, S1 and S4.

These values are found in the concentration limits obtained by: 3.254 mg/100 g (Kalkışım et al., 214), 1.02-3.98 mg/100 g (Polat, 2015), 2.21-4.32 mg/100 g (Yilmaz and Akça, 2017), 3.2, 3.8, 4.6 mg/100 g (Ebrhimi et al., 2017), 3.43-4.45 mg/100 g (Ni et al., 2016), 2.3-3.8 mg/100 g (Aryapak and Zirati, 2014), 3.356-4.892 mg/100 g (Ozyigit et al., 2018), 3.815-5.927 mg/100 g (Cosmulescu et al., 2009), 3.50 mg/100 g - kernel with yellow pellicle (Trandafir et al., 2016), 2.05-2.91 mg/100 g (Rada et al., 2018).

Zinc is an essential microelement that functions as a cofactor in almost 300 enzymes involved in the metabolism of proteins, carbohydrates, lipids, and energy metabolism [20]. Zinc also plays an important role in growth and cell division (where it is required for protein and DNA synthesis), in insulin activity, in the metabolism of the reproductive organs, and in liver function (Al-Fartusie and Mohssan, 2017). The Zn concentration in the analysed walnut kernel samples shows values between 1.23-3.58 mg/100 g, values that are found in the range of values obtained by 1.93-3.47 mg/100 g (Polat, 2015), 1.44-3.63 mg/100 g (Simsek, 2016), 1.97-5.48 mg/100 g (Yilmaz and Akça, 2017), 2.12, 2.2 and 3.8 mg/100 g (Ebrhimi et al., 2017), 1.48–2.77 mg/100 g (Ni et al., 2016), 2.98 and 2.96 mg/100 g (Trandafir et al., 2016), 1.606-2.615 mg/100 g (Rada et al., 2018). The best represented in terms of zinc content, without statistically significant differences, are the samples from sources S5 and S2. Lower concentrations of zinc, which can be considered statistically equal, have been identified in walnut kernels from sources S1, S3 and S4.

Copper is an essential element of several enzymes, such as cytochrome oxidase, monoamine oxidase, catalase, peroxidase, ascorbic acid oxidase, lactase, tyrosinase, and superoxide dismutase (Al-Fartusie and Mohssan, 2017). This essential micronutrient was determined in concentrates between 0.58-3.14 mg/100 g, values comparable to those reported: 0.5676-0.5494 mg/100 g (Ozgan et al., 2014), 1.173 mg/100 g (Kafaloglu, 2016), 0.65-1.43 mg/100 g (Polat, 2015), 0.77-2.44 mg/100 g (Simsek, 2016), 1.37-5.30 mg/100 g (Zhai, 2014), 0.93-2.47 mg/100 g (Ni Zhanglin et al., 2016), 0.521-2.08 mg/100 g (Cindrić, 2018), 1.41-3.223 and 2.93-347 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.964-1.007 mg/100 g (Rada et al., 2018), 2.14 and 2.03 mg/100 g (Trandafir et al., 2016).

The highest values of copper concentration, ensured statistically (significantly different statistically) were identified samples from sources S2 and S3. Lower statistically equal Cu concentrations were determined at sources S3 and S5. The walnut kernel from source S4 is the poorest in Zn.

Chromium, the essential microelement in the control of both blood glucose and fat levels (Marjan, 2013), was determined in the lowest concentrations of all the essential elements analysed. This trace element was determined in concentrations between 0.08-0.60 mg/100 g. Statistically, the walnut kernel samples richest in chromium, with no statistically significant differences, are those from S2 and S3 sources. Lower, statistically equal contents were identified in the walnut kernel from samples S1, S4 and S5.

Comparing the chromium concentrations obtained with those reported by: 0.1695-0.3323 mg/100 g (Özcan et al., 2010), 0.255-0.692 and 0.59-0.84 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.50 mg/100 g - walnut kernel with red pellicle (Trandafir et al., 2016) and 0.078-0.108 mg/100 g (Rada et al., 2018), no noticeable difference is observed.

The increased concentrations of mineral elements determined in the analyzed walnut kernel suggested the evaluation of the mineral intake of this valuable food. Practically, the degree of coverage of the daily mineral requirement corresponding to a consumption of 25 g of walnut kernel was determined (by calculation), for men and women aged between 19-50 years. 25 grams of walnut kernel were used on the recommendation of Seyit Mehmet Şen and Turan Karadeniz, who claim that in order to increase heart and brain health, 20-30 g of walnut kernel/day should be consumed from childhood (Şen and Karadeniz, 2015).

When assessing the mineral intake, the total content of the mineral elements present in 25 walnut kernels (mean values): 0.734 g Na, 99.70 g K, 22.45 g Ca, 52.80 g Mg, 1.473 g Mn, 0.858 g Fe, (0.588 g Zn, 0.434 g Cu, and 0.076 g Cr and the daily mining requirement recommended by the Food and Nutrition Board, Institute of Medicine, National (Food Academies, for people aged 19-50 years and Nutrition Board, Institute of Medicine, National Academies, for people aged 19-50 year) (Table 2).

To calculate the mineral intake, MI (Table 3) in the recommended daily diet, was performed using the relation: $MI\ [\%] = \frac{c}{a} \cdot 100$

where: MI - mineral intake, c-the amount of elements (mg) contained in the mass of walnut kernel consumed/day, a - the recommended amount of element (mg)/day.

Table 2. The recommended daily requirement and the upper tolerable limits for some mineral elements for the man and women 19-50 ages (Dietary reference intakes (DRIs): Recommended dietary allowances and adequate intakes, elements; Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements)

Specification	Raged People	Mineral element, mg/day								
		Na	K	As	Mg	Mn	Fe	Zn	Cu	Cr
Recommended values	Man	1500	3400	1000	420	2.3	8	11	0.9	0.035
	Women	1500	2600	1000	310	1.8	18	8	0.9	0.025
Tolerable values	Man	ND*	ND*	2500	350	11	45	40	0.010	ND*
	Women	ND*	ND*	2500	350	11	45	40	0.010	ND*

*Not determinable owing to a lack of a specific toxicological effect. ** WHO considered that chromium supplementation should not exceed 250 µg/day (WHO, 1996).

Table 3. Mineral content, in the recommended daily diet, for a consumption of 25 g walnut kernels

Source	People	Mineral supply (%)								
		Na	K	As	Mg	Mn	Fe	Zn	Cu	Cr
S1	Man	0.07	2.95	2.80	13.69	42.72	7.66	4.48	28.33	135.71
	Women	0.07	3.86	2.80	18.23	54.58	3.40	6.16	28.33	190.00
S2	Man	0.02	3.10	2.00	16.07	103.48	17.44	7.93	87.22	350.00
	Women	0.02	4.06	2.00	21.77	132.22	7.75	10.91	87.22	490.00
S3	Man	0.01	2.76	1.88	13.15	113.04	11.59	3.36	58.33	428.57
	Women	0.01	3.62	1.88	17.82	144.44	5.15	4.63	58.33	600.00
S4	Man	0.11	2.49	1.57	9.70	27.83	3.72	2.80	16.11	114.29
	Women	0.11	3.25	1.57	13.15	35.56	1.65	3.84	16.11	160.00
S5	Man	0.03	3.36	2.98	10.48	33.15	13.19	8.14	51.67	57.14
	Women	0.03	4.39	2.98	14.19	42.36	5.86	11.19	51.67	80.00
Mean	*Man*	*0.05*	*2.92*	*2.24*	*12.57*	*64.04*	*10.72*	*5.34*	*48.33*	*217.14*
	Women	*0.05*	*3.82*	*2.24*	*17.03*	*81.83*	*4.76*	*7.34*	*48.33*	*304.00*

As it can be seen from the table 3, a daily consumption of 25 g of walnut kernel contributes differently to ensuring the daily requirement of Na, K, Ca, Mg, Mn, Fe, Zn, Cu and Cr. The mineral intake, respectively the degree of coverage of the mineral requirement varies in wide limits between 0.01% (for Na - in men and women) - 600% (for Cr - in women), this being determined by the origin of the walnut kernel, the amount of kernel taken for consumption and its mineral concentration, the mineral requirement recommended by the category of consumers (men or women, age, pregnancy, lactation). Under the conditions of this experiment, the degree of coverage of the daily mineral requirement has the following values: 0.01-0.11% for Na, 2.49-3.36% for K, 1.57-2.98% for Ca, 9.70-16.07% for Mg, 27.83- 113.04% for Mn, 3.72-17.44% for Fe, 2.80-8.14% for Zn, 16.11-87.22% for Cu and 57.14-428.57% for Cr - in men and 0.01-0.11% for Na, 3.25-4.39% for K, 1.57 -2.98% for Ca, 13.15-21.77% for Mg, 35.56-144.443% for Mn, 1.65-7.75% for Fe, 3.84-11.19% for Zn, 16.11-87.22% for Cu and 80.00-600% for Cr.

Taking into account the average values of the concentrations of the analyzed mineral elements, it can be observed that the mineral contribution shows the following increasing trend: Na (0.05%) <Ca (2.24%) < K (2.92%) <Zn (5.34%) <Fe (10.72%) <Mg (12.57%) <Cu (48.22%) <Mn (64.04%) <Cr (217%) - for men and Na (0.05%) < Ca (2.24%) <K (3.82%) <Fe (4.76 %) <Zn (7.34%) <Mg (17.03%) <Cu (48.22%) <Mn (81.83%) < Cr (304%).

These values show that a consumption of 25 g of walnut kernel is not of interest from the point of view of Na, Ca and K, but can cover significant percentages of the need for Zn, Fe and Mg and high Cu and Mn. In addition, a consumption of 25 g of kernel ensures an amount of Cr three times higher than the recommended requirement. Because WHO considered that chromium supplementation should not exceed 250 μg/day (WHO, 1996) (Opinion of the Scientific Committee on Food on the Tolerable upper intake level of trivalent chromium), it can be observed that consumption of 25 g of walnut kernel does not present a risk of toxicity with Cr.

An eventual addition of the consumption of walnut kernels in order to increase the mineral intake may be possible, but with caution respecting tolerable upper intake levels elements corresponding to each element (Table 2). A supplement brings with it other compounds, which above certain concentration limits may have unwanted side effects.

CONCLUSIONS

The walnut kernel sold in agri-food markets in Timisoara (Romania) contains increased amounts of K, Mg, Ca, as well as significant amounts of Na, Fe, Mn, Zn, Cu and Cr unevenly distributed depending on the origin of the walnut kernel and the nature of the element analyzed.

The results obtained when evaluating the mineral intake show that a consumption of 25 g walnut kernel contributes differently to ensuring the daily mineral requirement depending on the nature of the element and the consumer

The analyzed walnut kernel is not of interest in terms of intake of Na, Ca and K, but can cover significant percentages of the need for Mg, Zn, Fe and high percentages of the daily requirement of Cr, Cu, and Mn.

Finally, it can be stated that the analyzed walnut kernel can be considered as an additional source especially of Cr, Mn, Cu, Mg, and to a lesser extent as a source of Zn and Fe.

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CLIMATE CHANGE AND THE NATURAL INFECTION OF APPLE TREES WITH *ERWINIA AMYLOVORA* BURRILL IN NORTHERN TRANSYLVANIA

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Abstract

Considering the high level of risks that infection with the bacteria Erwinia amylovora has in apple orchards in Romania, we have undertaken a four consecutive years (2019-2022) investigation in terms of fire blight occurrence. In the experimental plots typical symptoms, such as wilted or necrotic shoots in the form of 'shepherd's crook, were visible for two consecutive years (2021-2022), while flower clusters were not affected. The highest damage degree was observed in 2022, when was recorded the least amount of precipitation in the May-June period, but accompanied by extreme phenomena, such as strong winds. At the same time, during the study, the average daily temperatures in the case of May were the highest in 2022, respectively the second highest, in the case of June, being exceeded only by June 2019. According to our observations, the climate changes in the area, which consist of both the increase of the average annual temperature and the occurrence of some extreme phenomena with greater frequency, are favoring factors to increase the occurrence of Erwinia amylovora and amplify the fire blight symptoms in apples.

Key words: *average temperature, bacterial disease, fire blight, young shoots.*

INTRODUCTION

The apple (*Malus domestica* Borkh.) is one of the most consumed fruit in the world occupying the fourth position (Zhang et al., 2020) and the third in terms of quantity produced worldwide (FAOSTAT, 2024). In 2022, over 95 million tonnes were harvested across the globe (FAOSTAT, 2024). *Malus domestica* belongs to the Rosaceae family and is cultivated in all temperate regions of the world (Lyu et al., 2020). The cultivating area with apple has been growing ever since ancient times and it continues to increase even in the 21st century (Cen et al., 2020) (FAOSTAT, 2024).

Over the last 60 years, apple-growing technology changed from traditional production systems, using widely spaced large trees, to more intensive production systems, using trees closely spaced (Robinson, 2011). This was possible after the creation of the Malling series of dwarfing rootstocks (M.9 and M.26) but came with the disadvantage of an increased susceptibility to fire blight and some of the soil complex diseases (Goh et al., 2001). Another disadvantage is the appearance of more favorable conditions for the development of bacterial pathogens due to a bigger number of branch cuts, that create access points for pathogens as well as the young age of the trees in this type of plantation.

Fire blight is a devastating bacterial disease for a wide spectrum of fruit trees, in particular, cultural plants like apple, pear, and quince but also wild species belonging to the Rosaceae family. It affects more than 180 species of fruit trees and woody plants of the Rosaceae family (Vanneste et al., 2000), causing severe damage to flowers, fruits, fresh shoots, leaves, branches, trunk, and root collar (Aktepe et al., 2022).

The first mention of *Erwinia amylovora* infection as fire blight was made by Burrill in 1880 when he called this disease the anthrax of fruit trees (Burrill, 1880). The disease caused by *Erwinia amylovora* Burrill, was first time reported in 1780 on pear and quince in New York (United States) (Denning, 1974), then it spread worldwide to more than 60 countries

(Gaganidze et al., 2020; Santander et al., 2022). It is very difficult to control the disease because the bacterium has significant pathogenic capacity once it has entered the plant. Fire blight is one of the most damaging diseases of apples because of its destructiveness (Gaganidze et al., 2020).

Due to its high damage potential, *Erwinia amylovora* has had the status of a quarantine organism in many countries around the world. According to the latest information available on the website of EPPO (2024) this status has changed in many countries, such as Switzerland or some European Union members, because it no longer fulfill the criteria for being a quarantine organism. However, this is mainly due to the large area of distribution in those countries, not to a decrease in the disease importance and impact. The same status change also has occurred in Romania and the neighbouring countries. Nevertheless, there are countries such as Kazakhstan (Kairova et al., 2023) and Korea (Song et al., 2021) where *Erwinia amylovora* is still subject to quarantine.

As a result of global warming, the spread of fire blight to the unaffected countries and regions is inevitable (Gayder et al., 2023). In countries like Romania, where it is already present, there are concerns about how climate change is modifying the epidemiology of this disease. In Romania, climate change appears to consist of rising temperatures, amplification of extreme phenomena, and altered precipitation patterns according to the World Bank portal on climate (climateknoledgeportal.worldbank.org), and this pattern is expected to continue in the future.

Variations in environmental conditions are difficult to reproduce accurately and also they impact both the pathogenicity of bacteria and the response of plants to the aggressions of this pathogen (Van der Zwet and Beer 1999). This is also the reason why performing experiments that involve on-field observations is still a valuable tool for recommending the most efficient control strategies.

E. amylovora has high variability in damage occurrence patterns in the orchard because of the specific biology of this bacterium. *E. amylovora* transits from an epiphytic faze of colonization, on the plant surface, to an endophytic infection internally in the plant tissue (Zeng et al., 2021) when environmental conditions are favorable. However, the infection can become visible after 2 to 4 weeks after the epiphytic bacteria arrives in the orchard (Van der Zwet and Beer, 1999). Such behaviour can be one of the causes for high level epidemics even when the meteorological conditions are not optimal for infection.

These are the reasons why we undertaken a multi-year study regarding the natural infections with *Erwinia amylovora* in a temperate region affected by the climate change.

MATERIALS AND METHODS

The epidemiologic study was carried out in an experimental plot owned by the Fruit Research and Development Station (FRDS) Bistrita. It is situated at 47º10' North latitude and 24°30' East longitude in the northern part of Romania, on Bistrita hilly area, at 358 m above sea level. The climate has the characteristics of temperate-continental regions with hot summers and cold winters. The experimental plot consists of an untreated old apple orchard, established by using both Romanian and foreign apple cultivars and different apple hybrids. In this experimental plot, planting distances are 2 m x 4 m resulting a density of 1250 trees/ha. Observation were made on six apple cultivars: 'Auriu de Bistrita', 'Salva', 'Jonathan', 'Idared', 'Goldprim' and 'Generos'. Some of these cultivars are known to be susceptible to the infection with *E. amylovora*, such as 'Idared' (Sobieczewski et al., 2014) or 'Auriu de Bistrita' (Jakab-llyefalvi and Platon, 2012; Militaru et al., 2012), while others such us 'Goldprim' have a better behaviour, (Militaru et al., 2012).

The cultivars have been grafted on MM 106 rootstock and the trees were 28 years old when the survey has begun in 2019.

Visual observations have been performed weekly in the summer, from 2019 to 2022 and the symptoms displayed have been noted throughout the four consecutive years.

The meteorological data were graphically processed using the Microsoft Excel platform.

Temperature degree days were calculated as the difference between the average daily temperature and the value of 12.7 degrees Celsius which is the constant value, with the help of which it calculates day degrees for infections with *Erwinia amylovora*. The data thus obtained were summed up to be compared with the thresholds indicated in the MARYBLYT™ model (Steiner, 1990a, 1990b) for favorable conditions for *Erwinia amylovora* infections. Meteorological data were graphically analyzed using the Microsoft Excel platform.

The difference between the frequency of the symptoms on six cultivars during 2019-2022 period were statistically analysed using the One-Way Anova Test on the Microsoft Excel platform The statistical analysis was not continued with the Duncan test since because the differences were not significant.

RESULTS AND DISCUSSIONS

The year 2019 was characterized by the absence of fire blight symptoms on apple cultivars grown in our experimental plot. In all the other three years (2020, 2021, 2022) when the infection occurred, the fire blight symptoms were prevalent on shoots (Figure 1), by expressing the characteristic shepherd's crook shape. Rapidly growing shoots are the second known to be the next most susceptible organ of a plant to *Erwinia amylovora* after the flower (Van der Zwet and Beer, 1999).

Figure 1. Wilting of a shoot, caused by *Erwinia amylovora,* with bacterial exudate on 'Auriu de Bistrita', 22.06.2022. (Original photo)

Although regular inspections were carried out in the orchard both during the flowering period the growth and ripening of the fruits, no symptoms of bacterial fire were observed on the flower clusters and only sporadically on fruits.

Figure 2. Apple fruit infected with *Erwinia amylovora*, showing bacterial exudate forming from the lenticels, 22.06.2022. (Original photo)

The fact that the temperature has continuously increased in the Bistrita area may be a possible cause of the way the *Erwinia amylovora* epidemics manifested in recent years. In all cases, the primary infection appeared only on the shoots and not on the flowers, which has been observed in much southerly areas such as California (Van der Zwet and Beer, 1999).

The average annual temperature in the last 9 years has increased by up to 3 degrees Celsius (Figure 3) compared to the multiannual temperature recorded in the last century when it was 8.2°C in the Bistrita area (Crăciun et al., 1979).

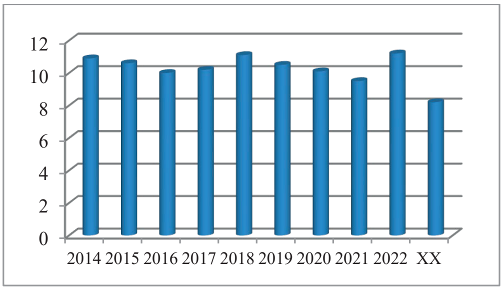

Figure 3. Average annual temperature in the period of 2014-2022 in comparison with XXth century.

The climatic changes in the northern part of Romania are very evident when it comes to the average monthly temperatures during winter months. The evolution of the average monthly temperatures, during November-February (2019-2022), can be seen in the chart of Figure 4. Since the winter temperature can have an impact on how the *Erwinia amylovora* epidemic manifests, We compared the new data to the multi-annual average of the last century (Crăciun et al., 1979). If in November of 2020, the average monthly temperature was the closest to the XXth century average with a difference of only 0.12ºC, in November of 2019, the temperature difference recorded was the largest in comparison to the last century, with a difference of 6.36ºC. Additionally, all other monthly averages were higher than the reference ones, as shown in Figure 4. These averages ranged from 0.74 to 5ºC.

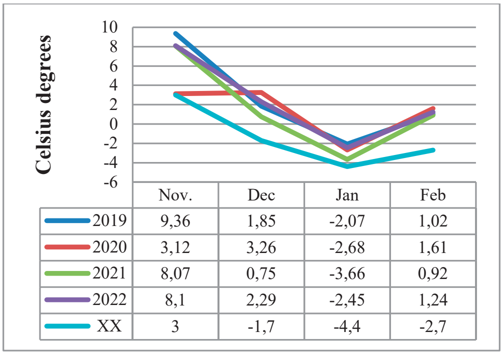

Figure 4. Average monthly temperature in winter months in 2019-2022 and the XXth century multiannual.

During the summer months we noticed that the highest average of monthly temperature in May was in 2022 (Figure 5), the year when *Erwinia amylovora* symptoms occurred with the highest frequency. While the highest average monthly temperature in June occurred in the year 2019, the lowest was recorded in June 2020 across all four experimental years. In all four consecutive years, the temperature exceeded the optimum threshold of 15.6°C for infections if the cumulative conditions for day degree (DD) temperature and humidity are met. Although June 2022 was not the warmest of the four years, the average monthly temperature was still high up to 20.17ºC. May and June were both warmer in 2019 and 2022, but despite these similarities, the way that fire blight manifested in the orchard was different.

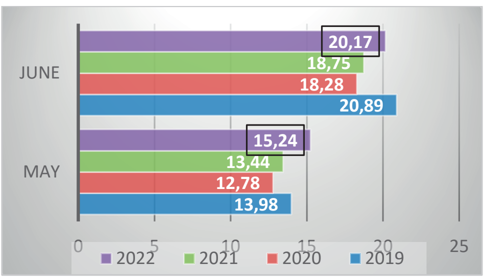

Figure 5. The average monthly temperature in May and June in Bistrita area from 2019 to2022 (ºC)

In June 2022, the average daily humidity was the lowest with a tendency of decreasing towards the end of the month (Figure 6). This possibly led to a more moderate evolution of *Erwinia amylovora* in the orchard. A higher humidity level might have led to a greater degree of damage in apples.

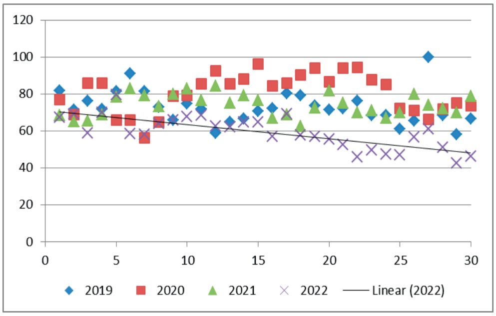

Figure 6. The average daily humidity (%) for the month of June, 2019-2022

Studying the daily average temperatures in May and June and calculating the sum of the day degree (DD) temperature according to the requirements of the MARYBLYT^{TM} system (Steiner, 1990a, 1990b), we found that in all 4 years, the thresholds for the initiation of infections, respectively for the appearance in the orchard, were exceeded only in June (Table 1). This is an explanation of the fact that the infections only manifested during the period of intense growth of shoots. In the Bistrita area, blossom usually occurs at the end of April or in the beginning of May, when the conditions for the initiation and manifestation of the *Erwinia amylovora* infection, according to MARYBLYT^{TM}, are not met.

Table 1. Cumulated day degrees (CDD) for initiation and for infection occurrence in the orchard in Bistrita area (2019-2022)

Year	∑ DD 12.7 ≥ 106	∑ DD12.7≥166
2019	10 June	16June
2020	15 June	26 June
2021	17 June	23 June
2022	04 June	13 June

Our results are rather different from those obtained by Jakab-llyefalvi and Platon (2012) regarding the appearance and manifestation of the attack of *Erwinia amylovora* on apple trees in the same area.

Unlike the results of the study from 2012, where the first symptoms were on flower clusters, in 2021 and 2022 the damage was present only on shoots. According to Van der Zwet and Beer (1999) this is a typical situation in some years, with optimum conditions for fire blight during the intense growing phase of the shoots and it produces high level damage in a very short period of time. Given the fact that the threshold for infection was surpassed in June in all experimental years during the rapid growth stage of the shoots, it is explainable why this happened.

There is another difference between 2022 and 2012 seasons due to the appearance of a second, smaller infection in most of the cultivars, in September 2022 (data not shown). In 2022, quince (*Cydonia oblonga*) faced a similar situation (Rosu-Mares et al., 2023), possibly due to large amount of precipitation fallen in August and September. It is notable that in the year with the highest rate of fire blight infections, June had the lowest amount of rainfall 18 l/m^2 of all the period analysed (Figure 7).

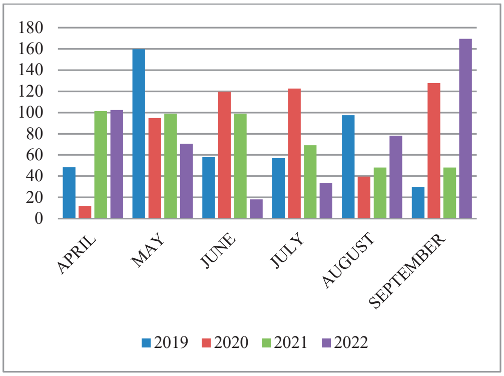

Figure 7. Rainfall (vertical axis, l/m^2) in 2019-2022 growing season, in Bistrita.

Another fact to point out is that the year 2022 had the highest number of rainy days in September and August of all four experimental years (Figure 8).

The frequency of fire blight symptoms occurring on six Romanian and foreign apple cultivars are presented in Figure 9. 'Auriu de Bistrita' was affected in 2021 and 2022, 'Salva' in 2020 and 2021 and 'Jonathan' in 2020 and 2022, while the others were damaged only in 2022.

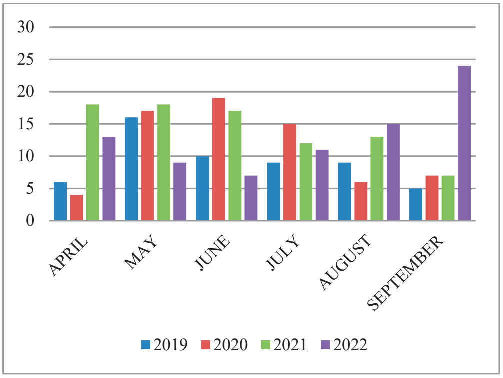

Figure 8. Number of rainy days (vertical axis) in 2019-2022 growing season, in Bistrita.

Although, the Romanian cultivar 'Auriu de Bistrita' suffered the most damage, confirming its susceptibility described by other previous researches (Jakab-llyefalvi and Platon, 2012; Militaru et al., 2012), the damage degrees in our study was lower than the ones mentioned in the other two previous experiments mentioned above.

Table 2. The frequency (F%) of fire blight symptoms on shoots of six untreated cultivars in Bistrita region, (2019 – 2022)

Cultivar/Year	2019	2020	2021	2022
'Auriu de Bistrita'	0	0	3.7	11.4
'Salva'	0	0.5	0.3	0
'Jonathan'	0	1.5	0	1
'Idared'	0	0	0	1.5
'Goldprim'	0	0	0	0.5
'Generos'	0	0	0	0.7

'Goldprim' and 'Generos' were the least affected cultivars by *Erwinia amylovora* followed by 'Idared'. The One-Way Anova Test performed on the data collected in 2019-2022 revealed that there are no significant differences between the cultivars reaction to the infection with *Erwinia amylovora* or between the years.

Overall data of our investigation revealed a lower level of damages caused by *Erwinia amylovora* to the six apple cultivars in the period 2019-2022 when compared with those previously reported Jakab-llyefalvi and Platon, 2012; Militaru et al., 2012). Considering the fact that the experimental plot was untreated, this is an encouraging result, suggesting that the pathogen *Erwinia amylovora* might become less aggressive in Bistrita region, once the climate changed as was shown.

CONCLUSIONS

The results of four years study revealed that the epidemiological evolution of fire blight in apple orchards varied from no infection in 2019 to a maximum in the year 2022. The results also suggests that the epidemiological patterns of *Erwinia amylovora* have changed and mostly affects young shoots.

This appears to be a consequence of climate changes in Bistrita area, which decreased the epidemicity of *Erwinia amylovora*.

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THE MINERAL PROFILE OF SOME PLUM (*PRUNUS DOMESTICA)* VARIETIES

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Abstract

This study aims to determine the concentration of some essential and toxic mineral elements from four plum varieties harvested from the orchard of the Lugoj area. The metallic elements were determined by the atomic absorption spectrometry method in flame. Phosphorus was determined in the form of molybdenum blue by spectrophotometric method. The obtained results show that the analysed plum varieties ('Record', 'Vinete românești', 'Čačanska lepotica' and 'Stanley') contain important quantities of essential elements unevenly distributed: 898 - 1.897 mg/kg K, 165 - 429 mg/kg Ca, 119 - 315 mg/kg Mg, 216 - 497 mg/kg P, 3.353 - 4.233 mg/kg Fe, 0.306- 0.726 mg/kg Mn, 0.306 - 1.488 mg/kg Zn, 0.780 - 2.088 mg/kg Cu, 0.032 - 0.198 mg/kg Cr and very small amounts below the lead limits. No significant amounts of Ni and Cd were detected. The results obtained following evaluation of the mineral intake show that, in the present conditions, the degree of coverage of the daily mineral requirement varies in wide limits, depending on the variety of fruit, consumer and the nature of the mineral element.

Key words*: mineral intake, minerals, Prunus domestica, varieties.*

INTRODUCTION

Prunus domestica is one of the most cultivated fruit trees in Romania, Serbia, France, Germany, Turkey and Italy (Konarska, 2015). The interest for the cultivation of this fruit tree is determined by the popularity of these fruits, which are particularly beneficial for human nutrition due to their low energy value and their increased content of nutrients and dietetics (Milošević & Milošević, 2012; Birwal et al., 2017**)**.

Plum fruits have been known and used as food by people since ancient times, either as fresh or dried fruit, or processed in the form of jams, compotes, jellies, candied fruits, as well as in the preparation of pastry etc. (Milošević & Milošević, 2012; Bozhkova, 2014).

The nutritional and dietary qualities of plums are determined by their nature and the concentration of compounds in their composition: carbohydrates (mono and disaccharides, pectin, dietary fiber), proteins, organic acids (citric acid, malic acid), phenolic compounds, flavonoids, carotenoids, vitamins, minerals, etc. (Milošević & Milošević, 2012; Birwal et al., 2017; Maglakelidze et al., 2017).

In addition to ensuring the nutritional level, eating plums helps prevent heart disease, lung and oral cancer, lowers blood sugar and blood pressure, helps with Alzheimer's disease, muscle degeneration, improves memory capacity, stimulates bone health, regulates the functioning of the digestive system, etc. (Heghedus et al., 2014; Maglakelidze et al, 2017**)**.

Plums are rich sources of macro and micro-elements: K, Ca, Mg, P, Na, Fe, Mn, Zn, Cu, Cr, etc., essential for important biochemical and physiological functions and are necessary for maintaining the body health (Grembecka & Szefer, 2013). The concentrations of plum mineral elements are influenced by a number of factors such as, varieties, pedo-climatic conditions, cultivation techniques and harvest period, the presence of pollution factors, etc. (Milošević et al., 2011; Miloševici & Miloševici, 2012; Grembecka & Szefer, 2013).

The fact that plums contain significant amounts of essential minerals and very small amounts of toxic metals is mentioned, not only by international databases. USDA Food Composition Databases (https://fdc.nal.usda.gov/fdc-app.html#/food-details/169949/nutrients) and Frida fooddata.dk. (https://frida.fooddata.dk/food/15?lang=en) (Table 1), but also a consistent number of studies by researchers in the field.

Table 1. Mineral profile of plum, after Frida fooddata.dk, and USDA Food Composition Databases

Mineral	Frida fooddata.dk		USDA Food Databases	
	Mean	Limits	Mean	Limits
Na, mg/100g	3.00	2.7 – 4	0	0 - 2
K, mg/100g	140	113 – 169	157	111-207
Ca, mg/100g	8.57	5.88 – 16	6.0	4.0-26
Mg, mg/100g	5.00	3.9 - 7.1	7	5- 9
P, mg/100g	19.6	14.3 - 25.6	16	9-25
Fe, mg/100g	0.140	0.05 - 0.2	0.17	0.07-0.59
Cu, mg/100g	0.0630	0.044 - 0.103	0.057	0.015- 0.151
Zn, mg/100g	0.0910	0.066 - 0.114	0.100	0.04- 0.3
I, μg/100g	0.400	0.15 - 0.6		-
Mn, mg/100g	0.1000	0.077 - 0.12	0.052	0.018-0.094
Cr, μg/100g	0.300	0 - 1.2	-	-
Se, μg/100g	0.114	0 - 0.7	0	0
Mo, μg/100g	0	0	-	-
Ni, μg/100g	6.67	0 - 22.3	-	-
Hg, μg/100g	0.0400	0 - 0.2	-	-
As, μg/100g	0.0200	0 - 0.1	-	-
Cd, μg/100g	0.0700	0 - 0.39	-	-
Pb, μg/100g	0.500	0.1 - 5	-	-

Particularly important information on the distribution of mineral elements in fresh fruit was reported by Grembecka and Szefer (2013), who analyzed 12 mineral elements from 98 fruits grown in different geographical areas of the world. The results show that plums (*Prunus domestica*) grown in Poland contain significant amounts of essential mineral elements.

Analyzing the influence of factors on the concentration of mineral elements in plums grown in Serbia, Milošević and Milošević (2012), found that the levels of ash and mineral elements in the analyzed plums, excepting nitrogen, differs significantly between the treatments performed. Similar values were obtained by Milošević et al. (2011), who analyzed plums grown in an area close to Čačak, which is a typical area for plum cultivation in Western Serbia. The results obtained from the analysis of fresh plums from seven promising F1 plum hybrids and their parents as a control cultivar shows that they contain significant amounts of minerals: 0.36-0.89% N, 0.04-0.09% P, 1.42-1.71% K, 0.05-0.09% Ca, 0.15-0.26% Mg, 0.42-0.49% Na, 19.25-23.65 mg/kg Fe of dry matter. The study authors report significant differences among hybrids, and among hybrids and control cultivars.

Analyzing several physical, pomological and nutritional properties of two plum (*Prunus domestica* L.) cultivars ('Stanley' and 'Frenze 90') grown in the Antalya region, (Ertekin et al., 2006) found that these fruits contain high amounts of essential minerals (of dry mater): 0.45% N, 870 ppm P, 1.200 ppm K, 230 ppm Mg, 160 ppm Na, 21.0 ppm Fe, 2.6 ppm Mn, 11.2 ppm Zn, 13 ppm Cu - in 'Stanley' plums and 0.62% N, 1.260 ppm P, 1.500 ppm K, 360 ppm Mg, 160 ppm Na, 20.0 ppm Fe, 3.6 ppm Mn, 14.7 ppm Zn, 46 ppm Cu - in 'Frenze' plums.

Data on the distribution of essential and toxic mineral elements in plums are presented in two papers by Heghedűş-Mîndru et al. (2014), who analysed some mineral in plum fruits purchased in supermarkets and food markets in Timisoara (Romania), as well as the concentration of some macro elements from plums grown in Rachita locality, Timis county (Romania) (Heghedűş-Mîndru et al., 2015). The results obtained from the analysis of plum samples purchased in supermarkets and food markets in Timisoara (Romania) show that the analysed plums contain significant quantities of essential elements and very low contents of toxic elements. It was observed that mineral elements with pronounced toxic potential Pb and Cd, have been identified in extremely small quantities, below the toxicity limits allowed by the European legislation (0.10 mg/kg, respectively 0.05 mg/kg) (https://eur-lex.europa.eu/legal-content/EN/TXT /PDF/?uri =CELEX:32015R 1005; http://extwprlegs1.fao.Org /docs/pdf /eur133613.pdf). Regarding the mineral concentration of plums grown in Rachita, the authors of the study determined important concentrations of macro elements (Heghedűş-Mîndru et al., 2015).

Data on the mineral composition of some plum varieties grown in Romania are presented by Bobis et al. (2017) which determined the chemical composition of 'HoneySweet' transgenic plum and two conventional ones: 'Reine Claude d'Althan' and 'Stanley'.

Preliminary results obtained in determining the mineral elements of plum varieties: 0.107-0.126 mg/kg Mg, 1.919-18.019 mg/kg Fe, 5.7.4-5.709 mg/kg Ca, 0.542-0.714 mg/kg Na and 1.936-3.364 mg/kg K, reveals high concentrations of Ca, K and Fe and lower concentrations of Na and Mg. 'Stanley' cultivar presented a very high amount of iron, but the highest amount of identified minerals from the three studied varieties.

In a study of the qualities of 'HoneySweet' cv., Ravelonandro et al. (2013) found that these fruits have nutritional qualities comparable to those of conventional plums.

The nutritional quality of plums is determined by a series of factors, like cultivated varieties and varieties, pedo-climatic conditions, but also cultivation techniques, etc. (Dimkova et al, 2018). Lombardi-Boccia et al. (2004) assessed aspects regarding the nutritional qualities of yellow plums (*Prunus domestica* L., var. 'Shiro'), conventionally and organically grown. The results obtained in determining some essential mineral elements in 'Shiro' cv. grown conventionally (chemical fertilizers were applied) and organic fertilizers (organic fertilizers were used): 11.9 mg/100 g P, 1.459 mg/100 g Na, 1.749 mg/100 g K, 4.909 mg/100 g Mg, 4.169 mg/100 g Ca, 0.299 mg/100 g Fe, 609 mg/100 g Zn, 609 mg/100 g Cu, 509 mg/100 g Mn - in conventionally grown plums and 14.6-15.1 mg/100 g P, 1.0-1.3 mg/100 g Na, 201-218 mg/100 g K, 5.40-5.80 mg/100 g Mg, 4.26-4.85 mg/100 g Ca, 0.26-0.27 mg/100 g Fe, 40-90 mg/100 g Zn, 50-60 mg/100 g Cu and 40-50 mg/100 g Mn - in organically grown plums, does not reveal major differences between the mode of culture. However, organic plums had slightly higher ash content (0.37%) than conventional ones (0.33%). Also, organically grown plums are richer in K, Mg and Zn compared to conventionally grown ones; Na and Cu were determined in higher concentrations in conventional cultivation.

Regarding the distribution of mineral elements in the edible part of plums, it was shown that there is a difference between mineral content in the skin and in the pulp (Cosmulescu et al., 2017; Motyleva et al., 2017). When analyzing the evaluation of the mineral profile in the skin and pulp of twelve varieties of plums grown in Romania, by the technique of spectrometry (ICP-MS), the study authors found significant differences between the concentrations in the two edible parts of fresh plums. Therefore, the peel of the plums has a higher mineral content compared to the pulp. Removing the skin from the fruit can lead to significant loss of mineral nutrients. This fact was also demonstrated by Motyleva et al., 2017 which when analyzing some macro and microelements from 6 cultivar of 3 plum species found that the values of Ca, Cu, Fe, Ni, Zn and Pb concentrations in plum skin is 2-10 times higher than in the pulp. The same is not true for potassium, which accumulates 7-20% more pulp.

It should be noted that the distribution of mineral elements in plums changes during their processing. This was reported by Yagmur and Taskin (2011), who analyzed the changes in the concentrations of some plum and strawberry mineral trace elements during their preservation: before washing, after washing treatment and the product final after heat treatment (canned fruit and syrup). The Fe, Cu, Zn and Mn concentrations determined in unprocessed plums were found to be, on average, 5.8389, 0.4510, 0.8630 and 0.5374 ppm, respectively. After washing, the concentrations of the mineral elements decreased to: 4.7032 ppm Fe, 0.4080 ppm Cu, 0.8119 ppm Zn and 0.4593 ppm Mn. The concentrations of the elements in canned plums decreased again to: 2.6112 ppm Fe, 0.3076 ppm Cu, 0.6780 ppm Zn and 0.4033 ppm Mn. It can be seen that the mineral values of plums have decreased during the preservation process. However, it was found that there was no significant difference between the mineral content of the fruit after washing and the combined total mineral values of the fruit and syrup after canning. From presented data, it can be seen that plums are important sources of essential minerals: K, Ca, Mg, Fe, Mn, Zn, etc. However, these fruits may also accidentally contain appreciable amounts of toxic elements (Pb, Cd et al.), or microelements that in certain concentrations present a risk of toxicity (Cr, Ni, Cu, etc.) (Papa et al., 2009). Analyzing the distribution of some minerals, separately from the pulp and skin, from five varieties of fruits and vegetables, Papa al. (2009), reported that the analyzed plums contain different amounts of toxic or potentially toxic elements (mg/kg dry weight): 0.31 (Pb), 0.12 (Cd), 5.49 (Cu), 0.11 (Cr), 1.30 (Ni) - in skin and 0.49 (Pb), 0.67(Cd), 6.48 (Cu), 0.46 (Cr), 1.10 (Ni) - in pulp (Papa et al., 2009). These values show higher mineral concentrates in the pulp than the skin. In addition, considering the fact that the concentrations of the analyzed elements are related to the dry mass, the concentrations of lead and cadmium in the plum pulp exceed the maximum limits provided by the legislation (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:320151005; http://extwprlegs1.fao.org/docs/pdf/eur133613.pdf). For this reason, we consider that to evaluate the nutritional qualities of these fruits, it is necessary to know, in equal measure, both the essential and toxicogenic elements.

This study aims to determine the concentrations of some essential elements (Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr) and toxic (Pb and Cd) from four plum cultivars: Romanian cvs. ('Record' and 'Vinete românești'), an American cv. ('Stanley') and a Serbian cv. ('Čačanska lepotica').

MATERIALS AND METHODS

Plant material

The plum samples were taken from the orchard Farm 3 located in the Lugoj area (Timis County, Romania), which belongs to the University of Life Sciences "King Mihai I" from Timisoara. The fruits come from four plum cultivars: 'Stanley', 'Vinete românești', 'Record' and 'Čačanska lepotica' and were harvested in the optimal ripening period (August, 2018). For each plum variety, 15 fruits were taken from different parts of the tree, which formed the basis for the analysis. After harvesting, the samples were immediately transported to the laboratory and stored (in polyethylene bags) cold at 2-4ºC until analysis. Before being analyzed, the fruits were washed with double-distilled water and dried by dabbing with filter paper. After removing the seeds, the fruit pulps were homogenized using a kitchen blender. Homogenized samples were immediately used for analysis.

Measurements

The determination of K, Ca, Mg, Fe, Mn, Zn, Cu, Cr, Ni, Pb and Cd was performed by flame atomic absorption spectrometry, using the method recommended and verified by Iordanescu et al. (2018). Basically, 3 g of plum sample was calcined at 550ºC in an oven for 8 hours (Nabertherm B150, Lilienthal, Germany). The ash resulting from calcination was solubilized in 20% HCl and made up to 20 ml in a volumetric flask. The mineral elements concerned were determined by atomic absorption spectroscopy (AAS; Varian 220 FAAS). Mix standard solutions (ICP Multi Element Standard solution IV CertiPUR) were purchased from Merck. Phosphorus was determined from the ash resulting from calcination, previously solubilized in 20% HCl and brought to a level of 100 ml, in the form of molybdenum blue by spectrophotometric method (Cintra 101 - UV-Visible Spectrometer, variant AA240 FS, at 715 nm) (Soceanu et al., 2009).

All chemicals and solvents used in this study were of analytical grade. The results were expressed on the basis of fresh weight (FW). Each value was the mean of three (n = 3) independent determinations.

Statistical analysis

The mean values and standard deviations of the concentrations of the mineral elements were calculated, and then they were statistically tested to find significant differences. The t-test was used: Two-Sample Assuming Unequal Variances for p=0.05 (IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSIONS

The results obtained in determining the total concentrations of micro and macro elements in the plum varieties tested are presented in Tables 2 and 3.

As can be seen from Tables 2, 3, the distribution of the analyzed mineral elements shows obvious nonhomogeneous, depending on the nature of the elements and the analyzed plum cultivar. The best represented are the macro elements: K, P, Ca and Mg, which represent over 99.00% of the total elements analyzed. The microelements: Fe, Mn, Zn, Cu, were determined in much lower and very small concentrations: Pb, Cr. Nickel, cadmium and chromium (in the Stanley and Record varieties) were not detected, their concentrations being below the limits of detection of the application. diminish much of their essentiality because the body functions require small amounts of such elements (small table of such elements.

Analyzing the total concentrations of the mineral elements analyzed in the four plum cultivars, it can be stated that the plums from the 'Čačanska lepotica' cv. have the highest content of mineral elements (3.146 mg/kg). The 'Vinete românești' (2.065 mg/kg) and 'Record' The lower concentrations of the essential microelements (Fe, Mn, Zn, Cu, Cr) do not (1.995 mg/kg) cultivars have lower contents compared to the 'Čačanska lepotica' cv., but are statistically close to each other (p>0.05). The lowest mineral content was determined by the 'Stanley' cv. (1.417 mg/kg), which shows obvious differences, especially in comparison with the 'Čačanska lepotica' cv., but also compared to the 'Vinete românești' and 'Record' cvs.

Table 2. The concentration of Na, K, Ca and Mg in some plum varieties (*Prunus domestica*)

Variety	Mineral element, mg/kg fresh fruit*			
	K	Ca	Mg	P
Stanley	898±11.52d	165±7.34c	119±4.97d	230±6.98c
Vinete românești	1.385±14.70b	174±6.16c	167±7.04b	331±9.42b
Record	1.332±11.67c	301±8.04b	134±5.35c	216±3.74c
Čačanska lepotica	1.897±13.37a	429±6.37a	315±5.72a	497±8.29a

*Different letters in same column show statistically significant differences between mean values at p<0.05

Table 3. The concentration of Fe, Mn, Zn, Cu, Cr, Ni, Pb and Cd in some plum varieties (*Prunus domestica*)

Variety	Mineral element, mg/kg fresh fruit							
	Fe	Mn	Zn	Cu	Cr	Ni	Pb	Cd
Stanley	3.432±0.38a	0.306 ±0.04b	0.750±0.09b	0.780±0.90b	SLD	SLD	0.027±0.01a	SLD
Vinete românești	3.353±0.21a	0.502±0.06a	1.488±0.09a	2.088±0.17a	0.198 ± 0.04a	SLD	0.042±0.01a	SLD
Record	4.215±0.31a	0.700±0.08a	1.173±0.15a	1.872±0.19a	SLD	SLD	0.019±0.01a	SLD
Čačanska lepotica	4.233±0.39a	0.726±0.08a	1.195±0.10a	1.943±0.35a	0.032 ± 0.01b	SLD	0.013±0.01a	SLD

*Different letters in same column show statistically significant differences between mean values at p<0.05

Potassium, an essential macro element that counteracts the effects of sodium, contributes to maintaining normal blood pressure and maintaining acid-base balance in the body (Pohl et al., 2013), was determined in the highest concentrations of all elements analyzed. The highest potassium concentrations were determined in the 'Čačanska lepotica' cv. (1.897 mg/kg). In lower but statistically different concentrations (p>0.05) it was identified in the 'Vinete românești' and 'Record' cvs.: 1.385, respectively 1.332 mg/kg. The poorest in potassium are the 'Stanley' cv. (898 mg/kg). It is difficult to compare the experimental results with the results obtained by other authors, because, as shown above, the distribution of mineral elements in plums varies widely depending on several factors: varieties, pedo-climatic conditions, techniques crop and harvest period, the presence of pollution factors, etc. In addition, the literature consulted contains too little data on the mineral profile of the plum varieties tested. However, if we consider the dry matter (DM) contents in the four plum cultivars (15.13% - 'Čačanska lepotica', 25.63% - 'Vinete românești', 12.09% - 'Stanley' and 22.20% - 'Record') determined by Scedei et al. (2019) and the values determined to be reported by other authors could be noticed possible differences. Thus the concentration of potassium in the 'Čačanska lepotica' cv. (12.538 mg/kg DM) is of the same order of magnitude as the potassium concentrations reported by Milošević & Milošević (2012), (15.500 mg/kg DM) and Milošević et al. (2011) (14.200-17.100 mg/kg DM) and relatively close to the concentration reported by Ertekin et al. (2006), (11.515 mg/kg DM) (Ertekin et al., 2006; Milošević et al., 2011; Milošević & Milošević, 2012). Potassium concentrations in the Romanian cultivars 'Stanley', 'Record' and 'Vinete românești' are below the levels reported by Milošević & Milošević (2012), Milošević et al. (2011) and Ertekin et al. (2006). Potassium concentration in the 'Čačanska lepotiča' cv. (1.897 mg/kg FM) is also reported in the concentration ranges reported by the USDA database (1.110-2.070 mg/kg FM) and of the same order of magnitude as the concentrations obtained by Cosmulescu et al. (2017) (1.5417 mg/kg FM), Božović et al. (2017) (1.893-2.199 mg/kg FM) and Lombardi-Boccia et al. (2004) (1.740 mg/kg FM). The concentration of potassium determined in the 'Stanley' cv. (898 mg/kg FM) is lower than that determined by Papa et al. (2009) (1.270 mg/kg FM).

Calcium, which functions as a component of bones and teeth, participates in the regulation of nerve and muscle function and plays a vital role in activating enzymes (Soetan et al., 2010) was determined in concentrations between 165-429 mg/kg. The distribution of calcium in the analyzed plum varieties is uneven. The highest concentrations of Ca, which differ statistically significantly ($P<0.05$) were determined in the varieties 'Čačanska lepotica' (429 mg/kg) and 'Record' (301 mg/kg). In much lower concentrations, which do not differ from each other from a statistical point of view, for $P<0.05$, they were determined in the 'Vinete românești' and 'Stanley' cvs.: 174, respectively 165 mg/kg. The concentration of calcium in 'Vinete românești' plums (679 mg/kg DM) shows values close to those of Milošević & Milošević (2012) (760 mg/kg DM) and Milošević et al. (2011) (780 mg/kg DM). Also, the 'Stanley' cv. contains almost identical amounts of Ca, compared to the Ca concentration determined by Ertekin et al. (2006) (1.202, compared to 1.200 mg/kg DM). The concentration of calcium in the varieties 'Stanley' (165 mg/kg FM) and 'Vinete românești' (174 mg/kg FM) falls within the limits reported in the USDA databases (40-260 mg/kg FM) and at the upper limit in Frida food database (58.8-160 mg/kg). In the varieties Record (1.356) and 'Čačanska lepotica' (2.835 mg/kg DM) higher amounts of Ca were determined than those determined by Milošević and Milošević (2012) (760 mg/kg DM) and Milošević et al. (2011) (780 mg/kg DM).

Magnesium is an essential macro element with many biological functions, where it functions as a cofactor in over 300 enzyme systems that regulate various biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control and blood pressure regulation (Falah et al., 2017). This element was determined in much lower concentrations than potassium and lower than phosphorus and calcium, the concentration limits being between 119 and 315 mg/kg. The richest in magnesium are the plums from the 'Čačanska lepotica' and 'Vinete românești' cvs. that contain statistically different amounts ($P<0.05$) of Mg: 315, respectively 167 ± 7.04 mg/kg. Lower, statistically different magnesium contents ($P<0.05$) were identified in the 'Record' (134 mg/kg) and 'Stanley' (119 mg/kg) cvs. The magnesium concentration determined in 'Stanley' plums (857 mg/kg DK) is lower than the concentrations determined by Milošević and Milošević (2012) (1.500 mg/kg DM) and Milošević et al. (2011) (1.500-2.600 mg/kg DM), but higher compared to the concentration reported by Ertekin et al. (2006) (230 mg/kg DM). The concentration of magnesium determined in plums of the 'Čačanska lepotica' cv. (2.835 mg/kg DM) is higher than the concentration determined by Milošević and Milošević (2012) (1.690 mg/kg DM), but it falls within the range of values determined by Milošević et al. (2011) (1.500-2.600 mg/kg DM). 'Record' (604 mg/kg DM) and 'Vinete românești' (652 mg/kg FM) cvs. have lower concentrations, compared to those determined by Milošević and Milošević (2012) (1.690 mg/kg DM) and Milošević N. et al. (2011) (1.500-2.600 mg/kg DM), but higher compared to the concentration reported by Ertekin et al. (2006) (230 mg/kg DM). Regarding the concentrations of magnesium, calculated from fresh substance, determined in the plums taken in the analysis, they are above the concentration levels reported by USDA databases (50-90 mg/kg FM), Frida food database (39-71) and Grembecka and Zviad (2013) (66.7-90.5 mg/kg FM).

Phosphorus, a macro element that functions as a component of bones, teeth, adenosine triphosphate, phosphorylated metabolic intermediates, and nucleic acids (Soetan et al., 2010), has been identified in concentrations ranging from 216 ± 3.74 to 497 mg/kg. The highest concentrations of phosphorus,

statistically different ($P<0.05$), were determined in the plums of the 'Čačanska lepotica' and 'Vinete românești' (497, respectively 331 mg/kg) cvs.. Much lower concentrations of phosphorus, statistically without differences between them ($P<0.05$) were identified in the cultivars 'Stanley' and 'Record': 230 ± 6.98, respectively 216 mg/kg.
Comparing the phosphorus concentrations determined in the analyzed plum cultivars (1.673-3.285 mg/kg DM, respectively 216-640 mg/kg FM) with those reported by Milošević and Milošević (2012) (520-640 mg/kg DM), Milošević et al. (2011) (400-900 mg/kg DM) and Ertekin et al. (2006) (870 mg/kg DM), respectively with USDA databases (90-250 mg/kg FM) and Frida food database (520-640 mg/kg FM) can be seen to have higher values.
Iron is an essential trace element, which is an important component of hemoglobin, an erythrocyte protein that transfers oxygen from the lungs to tissues (Falah et al., 2017) in the highest concentrations of the analyzed trace elements. The concentration of iron in the four plum cultivars was 3.432 ('Stanley'), 3.53 ('Vinete românești'), 4.215 ('Record'), respectively 4.233 mg/kg ('Čačanska lepotica'), shows very close values and statistically equal for $P<0.05$. Iron concentrations in the varieties determined in 'Čačanska lepotica' (27.978 mg/kg DM) and 'Stanley' (24.996 mg/kg DM) are higher than those obtained by Milošević and Milošević (2012) (19.25 mg/kg DM - in 'Čačanska lepotica' and 18.25 mg/kg DM - in 'Stanley'), Milošević N. et al. (2011) (19.25 - 23.25 mg/kg DM, in hybrid 'Čačanska lepotica/Stanley') and Ertekin et al. (2006) (21.0 mg/kg DM - in 'Stanley'). The concentration of iron in the cultivars 'Record' (18.98625 mg/kg DM) and 'Vinete românești' (13.982 mg/kg DM) is below the concentration reports reported by those obtained by Milošević and Milošević (2012) (18.0 - 21.62 mg/kg DM). Comparing the concentrations of iron, reported from fresh substance (3.353-4.233 mg/kg FM) with the values recorded in the consulted databases, the experimental values are lower than those recorded in Frida food database (0.5-2.0 mg/kg FM), but falls within the limits of the USDA database (0.7-5.9 mg/kg FM). The iron concentrations of the analyzed plum varieties are higher than those determined by Djina Božović et al. (2013) (1.2-1.95 mg/kg FM) and Lombardi-Boccia G. et al. (2004) (2.9 mg/kg FM), but lower than those reported by Yagmur and Taskın (2011) (5.8389 mg/kg FM) and Grembecka and Szefer (2013) (4.4-7.7 mg/kg FM).
Manganese microelement that functions both as an activator and as a constituent of several enzymes in the body (Mehri, 2020) was determined in concentrations between 0.303-0.7026 mg/kg. In higher concentrations, statistically equal ($P<0.05$), it was determined in the varieties 'Čačanska lepotica' (0.726 mg/kg), 'Record' (0.700 mg/kg) and 'Vinete românești' (0.502 mg/kg). Statistically lower concentrations of Mn ($P<0.05$) compared to other varieties were determined in the 'Stanley' cv. (0.306 mg/kg). The manganese concentrations determined in the plum varieties 'Čačanska lepotica' (4.798 mg/kg DM) and 'Stanley' (2.227 mg/kg DM) are lower than those obtained by Milošević and Milošević (2012) (12.50, respectively 10.62 mg/kg DM), but close to the concentration determined by Ertekin et al. (2006) (2.6 mg/kg DM) in 'Stanley' plums. It can also be seen that the fresh plum varieties analyzed contain more manganese (0.306-0.700 mg/kg FM) compared to the limits contained in the Frida food database (0.77-1.20 mg/kg FM) and USDA databases (0.18-0.94 mg/kg FM) and close to the manganese content determined by Yagmur and Taskın (2012) (0.5374 mg/kg FM).
Zinc is a microelement involved in many enzymes or as a stabilizer of the molecular structure of constituents and subcellular membranes (Falah et al., 2017). This element that participates in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids and that plays an essential role in the transcription and translation of polynucleotides and therefore in the processes of genetic expression (Mehri, 2020) was determined in different concentrations between 0.750 mg/kg and 1.488 mg/kg. The poorest in zinc is the 'Stanley' cv., where it was identified in the lowest concentration and statistically different ($P<0.05$) from the other three analyzed varieties. Richer in zinc are the cultivars 'Vinete românești', 'Čačanska

lepotica' and 'Record', where it was identified in close and statistically equal concentrations ($P<0.05$): 1.488, 1.195, respectively 1.173 mg/kg. As with manganese, the concentrations of zinc in the cultivars 'Čačanska lepotica' (7.980 mg/kg DM) and 'Stanley' (5.462 mg/kg DM) are lower than those obtained by Milošević and Milošević (2012) (20.25, respectively 19.66 mg/kg DM) and Ertekin et al. (2006) (11.2 mg/kg DM - in 'Stanley' plums). Comparing the Mn concentrations of the analyzed fresh plum varieties (0.750-1.488 mg/kg FM) with the limits contained in the databases, it can be seen that they are very close to the values recorded in Frida food database (0.66-1.14 mg/kg FM) and falls within the limits of the USDA databases (0.4-3.0 mg/kg FM). Comparing the concentrations of 'Čačanska lepotica' (1.195 mg/kg FM) and 'Stanley' analyzed (0.750 mg/kg FM) varieties with the concentrations determined by Djina Božović et al. (2017) (0.410 mg/kg FM - in 'Čačanska lepotica' and 0.550 mg/kg FM - in 'Stanle'y) there are differences in the 'Čačanska lepotica' cv. and similar values in the 'Stanley' cv.

Copper an essential micronutrient that plays an important role as a cofactor for several cellular processes, the deficiency of which leads to anemia and bone marrow suppression, followed by a neurological syndrome called myelopathy (Davarynejad et al., 2012), was determined in concentrations lower than iron and relatively close to the concentration of zinc. The lowest amount of copper (0.780 mg/kg), different statistically ($P<0.05$) compared to other varieties is recorded in the case of the 'Stanley' cv. Close, statistically equal values ($P<0.05$) of Cu content were determined in the 'Vinete românești' cv. (2.088 mg/kg), 'Čačanska lepotica' (1.943 mg/kg) and 'Record' (1.872 mg/kg). Comparing the copper concentrations obtained experimentally (5.687-12.842 mg/kg DM) with those recorded in the consulted studies can be shown as higher than those obtained by Milošević and Milošević (2012) (2.750-3.775 mg/kg DM), but lower than those reported by Ertekin et al. (2006) (13.80 in 'Stanley' mg/kg DM) and Hegedus-Mandru et al. (2014) (15.0 mg/kg FM). With the exception of 'Stanley' plums (in which the Cu concentration is 0.780 mg/kg FM), the experimentally determined copper concentrations are higher than those determined by Grembecka and Szefer (2013) (0.30-0.70 mg/kg FM), Lombardi-Boccia et al. (2004) (0.600 mg/kg FM), Yagmur and Taskın (2012) (0.451 mg/kg FM) and those recorded in Frida food database (0.74-1.03 mg/kg FM) and USDA databases (0.15-1.51 mg/kg FM).

Nickel is considered an essential microelement, although its biological function in the human body is still somewhat unclear. Since nickel is found in the body in higher concentrations of nucleic acids, especially RNA, it is believed to be involved in some way in the structure or function of proteins (Mehri, 2020). The concentrations of this element in the analyzed plum varieties are below the detection limits, under the conditions of the present experiment.

Chromium, an essential microelement that potentiates insulin, thus influencing the metabolism of carbohydrates, lipids and proteins (Mehri, 2020), was determined in significant concentrations, only in the 'Vinete românești' and 'Čačanska lepotica' cvs.: 0.198 and 0.032 mg/kg, respectively. These concentrations show values close to the value reported by Grembecka and Szefer (2013) (0.04 - 0.1 mg/kg FM) and compared to those recorded in Frida databases (0-0.12 mg/kg FM).

Lead is an extremely toxic metal that affects almost every organ in the body, the most affected being the nervous system which may contribute to behavioral problems, learning deficits, and lowered IQ (Mehri, 2020). The concentrations of this heavy metal in all four plum cultivars show very low and statistically equal values, for $P<0.050$, below the allowed toxicity limits (0.1 mg/kg) (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R1005).

Cadmium is considered a toxic and hazardous metal to both humans and animals, which acts as a mitogen and promotes cancer in several tissues (Sharma et al., 2015). This heavy metal with pronounced toxicity, the concentration of which in fresh fruit (http://extwprlegs1.fao.org/docs/pdf/eur133613.pdf), has not been determined under the experimental conditions described. The experimental results obtained in determining the essential elements from the fresh plums taken in the experiment allowed the evaluation of the mineral intake of these fruits. To evaluate the mineral intake, the recommended mineral requirement in the daily diet was taken into account (Table 4), and the mineral content from the mineral canteen in the mass of consumed fruit (Table 5).

Table 4. Dietary reference intakes (DRIs): Recommended dietary allowances and elements tolerable upper intake levels elements for the man and women 19-50 ages

Values	Gender	Mineral element, mg/day								
		K	Ca	Mg	P	Mn	Fe	Zn	Cu	Cr**
Recommended	Man	3.400	1.000	420	700	2.3	8	11	0.9	0.035
	Women	2.600	1.000	310	700	1.8	18	8	0.9	0.025
Tolerable	Man	ND*	2500	350	4.000	11	45	40	10	ND*
	Women	ND*	2500	350	3.000	11	45	40	10	ND*

*ND: Not determinable owing to a lack of a specific toxicological effect. WHO considered that chromium supplementation should not exceed 250 µg/day (Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium (expressed April 4, 2003), https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out197_en.pdf).
(Dietary reference intakes (DRIs): Recommended dietary allowances and adequate intakes, elements, http://nationalacademies.org/hmd/~/media/Files/Report%20Files/2019/ DRI-Tables-2019/2_RDAAIVVE.pdf?la=en; Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements, https://www.ncbi.nlm.nih.gov/books/NBK545442/table/appJ_tab9/?report=objectonly)

The correlation analysis (Table 5) shows very good correlations (r>0.9) between the pairs: Mg/K (r = 0.92), Fe/Ca (0.91), P/Mg (0.98) and Cu/Zn (0.92). Good correlations (r> 0.8) can also be observed between the pairs: Ca/K (0.86), P/Ca (0.88), Mn/K (0.84), Mg/Ca (0.83), Mn/Ca (0.86) and Mn/Fe (0.88).

Table 5. Matrix of correlations between macro and microelements

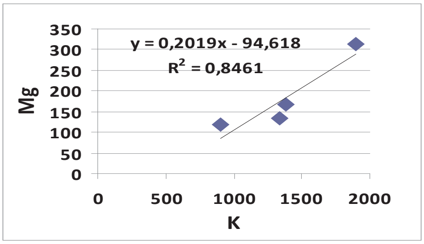
	K	Ca	Mg	P	Fe	Mn	Zn	Cu
K	1							
Ca	**0.856651**	1						
Mg	***0.919843***	0.828323	1					
P	**0.879955**	0.688435	***0.975513***	1				
Fe	0.642216	***0.909385***	0.521993	0.330156	1			
Mn	**0.841769**	**0.863104**	0.617297	0.493218	**0.881082**	1		
Zn	0.587299	0.141395	0.329969	0.420533	0.018527	0.489148	1	
Cu	0.763752	0.459788	0.471624	0.479935	0.395917	0.782478	***0.924732***	1

Thus, for strong correlations, the regression curves can make a forecast of the evolution of the content of an element in relation to the concentration of the other analyzed elements (Figure 1: a, b, c, d).

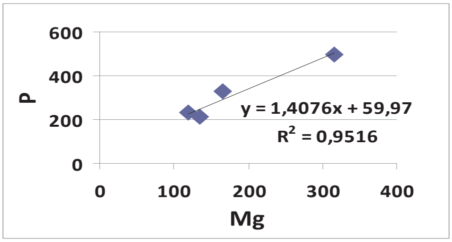
The experimental results obtained for the determination of the essential elements in the fresh plums taken in the experiment allowed the evaluation of the mineral intake of these fruits. For the evaluation of the mineral intake, the recommended mineral requirement in the daily diet (Table 5) and the mineral content of the mineral canteen in the consumed fruit mass (Table 6) were considered.

From those presented in Table 7 it can be seen that the degree of coverage of the daily requirement of mineral elements, at a consumption of 400 g of fresh plum pulp is uneven, depending on the consumer, the nature of the mineral element and the variety of and presents values included in the following limitation: 4.68-22.32% K, 6.60-17.16% Ca, 8.45-30.00% Mg, 12.34-28.40% P, 16.77-21.17% Fe, 5.32-12.63% Mn, 2.73-5.41% Zn, 34.67-92.80% Cu and 36.57-226% Cr - for men's 6.12-21.31% K, 6.60-17.16% Ca, 11.45-40.65% Mg, 12.34-28.40% P, 7.45-9.41% Fe, 6.80-16.13% Mn, 3.75-7.44% Zn, 34.67-92.80% Cu and 51.20-317% Cr - for women.

A hierarchy of fruit varieties according to their mineral intake is quite difficult, as the distribution of mineral elements in the analyzed fruit varieties is different. However, considering the sum of the total contents of each fruit variety, which shows the following decreasing trend: 3146 mg/kg 'Čačanska lepotica' plums; >2065 mg/kg 'Vinete românești' plums; >995 mg/kg 'Record' plums; >1417 mg/kg 'Stanley' plums, it can be stated that in this order also decreases the mineral intake of these fruits. The average inputs with mineral elements, for which the average of the individual values of the four fruit varieties were taken into account, show the following decreasing trend (%): Cu>Fe>P>Mg>K>Ca>Mn>Zn – men's Cu>Mg>K>P>Mn>Ca>Fe>Zn - women.



a



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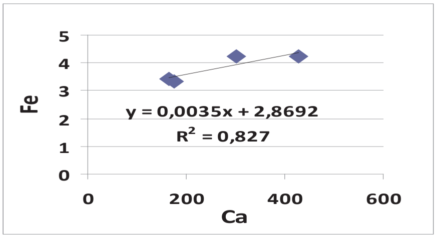

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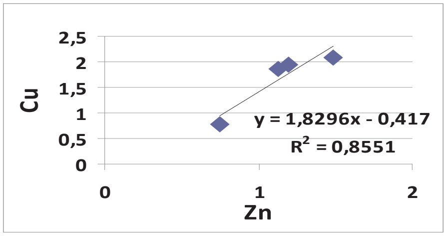

d

Figure 1. Regression lines for pairs of elements: Mg/K (a), P/Mg (b), Fe/Ca (c) and Cu/Zn (d)

Table 6. Dietary reference intakes (DRIs): Recommended dietary allowances and elements tolerable upper intake levels elements for the man and women 19-50 ages

Values	People range	Mineral element, mg/day								
		K	Ca	Mg	P	Mn	Fe	Zn	Cu	Cr**
Recommended	Man	3.400	1.000	420	700	2.3	8	11	0.9	0.035
	Women	2.600	1.000	310	700	1.8	18	8	0.9	0.025
Tolerable	Man	ND*	2.500	350	4.000	11	45	40	10	ND*
	Women	ND*	2.500	350	3.000	11	45	40	10	ND*

*ND: Not determinable owing to a lack of a specific toxicological effect. WHO considered that chromium supplementation should not exceed 250 µg/day (https://www.ncbi.nlm.nih.gov/books/NBK545442/table/appJ_tab9/?report=objectonly; https://ec.europa.eu/food/sites/food/files/safety/docs/ sci -com_scf_out197en.pdf)

Table 7. Mineral intake in the recommended daily diet (men and women aged between 19 and 50 years)

Variety		K	Ca	Mg	P	Fe	Mn	Zn	Cu	Cr
Stanley	M	4.68	6.60	8.45	13.14	17.16	5.32	2.73	34.67	-
	W	6.12	6.60	11.45	13.14	7.63	6.80	3.75	34.67	-
Vinete românești	M	16.29	6.96	15.90	18.91	16.77	8.73	5.41	92.80	226
	W	21.31	6.96	21.55	18.91	7.45	11.16	7.44	92.80	317
Record	M	15.72	12.04	12.76	12.34	21.08	12.17	4.27	83.20	-
	W	20.55	12.04	17.29	12.34	9.37	15.56	5.87	83.20	-
Čačanska lepotica	M	22.32	17.16	30.00	28.40	21.17	12.63	4.35	86.36	36.57
	W	29.18	17.16	40.65	28.40	9.41	16.13	5.98	86.36	51.20
Mean values	**M**	***14.75***	***10.69***	***16.78***	***18.20***	***19.04***	***9.71***	***4.19***	***74.26***	**-**
	W	***19.57***	***10.07***	***22.73***	***18.20***	***8.46***	***12.41***	***5.76***	***74.26***	**-**

A possible supplement of plum consumption, to increase the mineral intake may be possible but with caution respecting the tolerable quantities for each element (Table 7). In addition, it should be borne in mind that an increase brings with it other compounds, which in too large quantities can have undesirable side effects.

CONCLUSIONS

The cultivars of *Prunus domestica*: 'Stanley', 'Vinete românești', 'Record' and 'Čačanska lepotica' taken in the experiment contain significant quantities of essential mineral elements unevenly distributed depending on the variety and the nature of the element analyzed: 898-1.897 mg/kg K, 165-429 mg/kg Ca, 119-315 mg/kg Mg, 216-497 mg/kg P, 3.353-4.233 mg/kg Fe, 0.306-0.726 mg/kg Mn, 0.306-1.488 mg/kg Zn, 0.780-2.088 mg/kg Cu, 0.032-0.198 mg/kg Cr and low amounts, below the toxicity limits of Pb toxic elements (0.013-0.042 mg/kg). No significant amounts of Ni and Cd were detected.

The highest mineral content (sum of mineral elements) was determined in the 'Čačanska lepotica' cv. (3.146 mg/kg), followed by the 'Vinete românești' (2.065 mg/kg) and 'Record' (1.995 mg/kg) cvs., which have higher contents, small compared to the 'Čačanska lepotica' cv., but close to each other, but statistically different ($p<0.05$). The lowest mineral content was determined by the 'Stanley' cv. (1.417 mg/kg), which shows obvious differences, especially in comparison with the 'Čačanska lepotica' cv., but also compared to the 'Vinete românești' and 'Record' cvs. Mutual correlations were determined between mineral elements, which can prove dependence between macro and microelements. The highest correlation coefficient values were determined for the elemental pairs: Mg/K, Ca/Fe, Mg/P and Zn/Cu ($r> 0.9$) and K/Ca, K/P, K/Mn, Ca/Mg, Ca/Mn and Fe/Mn ($r> 0.8$).

Under the conditions of the present experiment to evaluate the mineral intake, the degree of coverage of the daily requirement of mineral elements presents different values depending on the consumer gender (male or female), the variety and the nature of the mineral element. The average inputs with mineral elements, for which the average of the individual values of the four fruit varieties were considered, show the following decreasing trend. (%): Cu> Fe> P> Mg> K> Ca> Mn> Zn - man and Cu> Mg> K> P> Mn> Ca> Fe> Zn - woman.

As a conclusion it can be stated that the plums taken in the experiment contain important quantities of essential mineral elements and can be considered in respect of mineral intake. In addition, these fruits are not contaminated with lead or cadmium.

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MORPHOLOGICAL VARIABILITY OF SOME ROSEHIP FRUITS (*ROSA CANINA* L.) FROM THE SPONTANEOUS FLORA OF OLT COUNTY, ROMANIA

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Abstract

The present work aimed to analyse the variability of some genotypes of Rosa canina L. from some forest ecosystems located in the southern area of Olt County. Nine rosehip genotypes were subjected to the study, in which the morphological variability of the fruit was analysed starting from the dimensional and weight characteristics. It was found that there is a high variability between genotypes, highlighted by the Coefficient of Variability for weight (CV% = 32.86), height (CV% = 12.61), small fruit diameter (CV% = 12.47), and large fruit diameter (CV% = 12.33). The high existing variability is an essential factor through the presence of these genotypes and their usefulness as a sustainable resource in diversifying the fruit tree material.

Key words*: rosehip, fruits, genotypes, variability, coefficient of variation.*

INTRODUCTION

Wild edible fruit species, including those of the genus *Rosa*, are found naturally in many parts of the world, especially in rural areas and forests, and exhibit great morphological and biochemical diversity (Eyduran et al., 2022; Stoenescu et al., 2022). *Rosa* genus is one of the most diverse vegetable genera, fruit plants of this genus have been used for centuries (Bozhuyuk et al., 2021). Rosehip (*Rosa canina* L.) is a thorny shrub, pentaploid (2n=35), and xeromesophyte, reaching heights between 3 and 5 m (Ghiorghiță et al., 2012a, 2012b, 2012c). Taxonomically it is included in *Rosales* order, *Rosaceae* family, *Rosoidae* subfamily, and *Rosa* L. genus comprising 100-250 species (Šindrak et al., 2012; Stoenescu & Cosmulescu, 2021). The species is native to Europe, North-East Africa, and Western Asia and can be found from 0 to 1200 m altitude (Ghiorghiță et al., 2012a, 2012b; Tomljenović et al., 2022). Rosehip has great diversity (Cosmulescu et al., 2022); more than 200 varieties, forms and hybrids have been identified (Ghiorghiță et al., 2012c). This shrub provides food and habitat for some animals, while in horticulture, it is used as a rootstock for ornamental varieties of roses (Šindrak et al., 2012), it is one of the most widely used non-wood forest products (Güler et al., 2021). It can be planted to prevent soil erosion, as it is adaptable to different soils and environmental conditions. Few diseases or pests can seriously damage the adult plant (Šindrak et al., 2012). The fruit has a brick-red colour, to an intense red, with a pleasantly sour taste, and inside there are numerous small and hairy achenes (Iancu et al., 2020). Due to its proven effects on immunity, rosehip fruits quickly found a common usefulness, being found both in food (Eldaw & Çiftci, 2023) and in some medicines; the plant can also be found in feed, fuel, agriculture, tools, forest curtains, and even as a ritual element (Bozhuyuk et al., 2021). Traditionally, rosehips are used against many diseases due to their immunosuppressive, antioxidant, anti-inflammatory, antiarthritic, analgesic, antidiabetic, cardioprotective, antinociceptive, antimicrobial, gastroprotective, and skin ameliorative effects (Teodorescu et al., 2023). Unlike cultivated varieties, these wild fruits are famous for their intense aroma and fragrance, but they also show greater resistance to adverse soil and climatic conditions (Eyduran et al., 2022). The present paper aims to carry out an analysis of the variability of *Rosa canina* L. genotypes in some forest ecosystems located in the southern area of Olt County in order to develop and complete morphological knowledge regarding this species.

MATERIALS AND METHODS

The rosehip fruits (*Rosae fructus*) were harvested from plants of spontaneous flora in the south of Olt County, in the vicinity of forests located on the administrative territory of Vlădila, Studina and Grădinile communes. From the multitude of genotypes identified and analysed, using specific methods (fruit quality, productivity, disease resistance), nine genotypes were selected for analysis. They were located by GPS coordinates (Table 1) for further observation. For the coding of individuals, two letters represent the harvesting area (GR, ST; VL), two letters symbolize the species (RS), and a number. Vlădila Forest and Studinița Forest are protected areas and can also be identified by site names such as ROSCI0183 and ROSCI0174 respectively. Grădinile forest is located, according to Iancu & Iancu (2017), in the east, southeast, and northwest of the rural settlement.

Table 1. Encoding and GPS coordinates of identified *Rosa canina* genotypes

Genotype	GPS coordinates
GR RC 01	43°56'23"N 24°23'43"E
GR RC 02	43°56'31"N 24°24'03"E
GR RC 03	43°56'26"N 24°23'53"E
ST RC 01	43°58'14"N 24°24'50"E
ST RC 02	43°58'35"N 24°24'29"E
ST RC 03	43°58'36"N 24°24'21"E
VL RC 01	44°00'13"N 24°22'58"E
VL RC 02	44°00'21"N 24°21'56"E
VL RC 03	44°00'27"N 24°21'08"E

The climatic conditions of the three zones are similar since the distances between the studied forest habitats range from 2 to 10 km. Geographically, the studied forest habitats are located at the border between the Caracal Plain and the Leu-Rotunda High Field. The climate is forest-steppe, dry due to the unevenness of precipitation in the growing season and high temperatures (Stamin & Olaru, 2023). From a hydrological point of view, Vlădila Forest is crossed by Vlădila Creek, on which three reservoirs were built; in the case of Studinița Forest the nearest water source is Studina Pond at a distance of about 500 m, and the forests in Grădinile commune are near the Grădinile Creek and the three ponds of anthropogenic origin (Iancu & Iancu, 2017) located on the administrative territory. The soils mainly belong to the group of mollisols with cambic chernozems and clayey chernozems (Olaru & Cheptănariu, 2023).

Observations were made for 100 fruits of each genotype studied. With the help of the electronic caliper, the height of the fruit and two diameters, respectively, were measured in the middle area; taking into account that the fruit does not have a perfect shape, it was aimed that the two diameters are approximately perpendicular. The weight of each fruit was also determined using the analytical balance.

The obtained data were statistically processed through Microsoft Excel 2010, determining the mean value for each characteristic, genotype (X), standard deviation (SD), coefficient of variation (CV% = SD/X*100), histograms of measured parameters, and correlations between parameters.

RESULTS AND DISCUSSIONS

The values obtained for the morphological characteristics of the fruit are given in Table 2. The physical characteristics of fruits are a criterion for selecting valuable genotypes. The size and shape of the fruit determine the market. They vary both within the genotype and from genotype to genotype within the population. Regarding the height of the fruits, it can be found that the lowest measured value was 10.47 mm (VL RC 01), and the highest recorded height value was 27.68 mm (VL RC 02). In the study of Rosu et al. (2011), the limits of variation for rosehip were between 11.4 and 30.9 mm, similar to those obtained in this study. The average value for fruit height was 20.02 mm, with limits ranging from 21.86 mm (GR RC 02) to 18.34 mm (GR RC 01). The obtained results are comparable with the data presented by Rosu et al. (2011), for genotypes from Moldova area (16.8 and 24.0 mm). The coefficient of variation for all genotypes was 12.61%, which shows a lower variability compared to the research conducted by Stoenescu & Cosmulescu (2021), where the coefficient of variation was 16.47%, the genotypes being collected from another area of Oltenia, than the one in the present study.

Table 2. Morphological characteristics of the studied rosehips genotype

Genotype	Descriptive statistics	Fruit height (mm)	Small fruit diameter (mm)	Large fruit diameter (mm)	Fruit weight (g)
GR RC 01	X ± SD	18.34 ± 1.69	10.54 ± 0.92	10.71 ± 0.89	1.01±0.24
	Variations limit	14.52 - 21.69	8 - 13.51	8.45 - 13.69	0.55 - 1.74
	CV %	9.25	8.80	8.36	23.72
GR RC 02	X ± SD	21.86 ± 2.24	12.06 ± 1.26	12.34 ± 1.28	1.80 ± 0.54
	Variations limit	11.97 - 26.90	9.24 - 15.17	9.48 - 15.32	0.76 - 3.13
	CV %	10.28	10.47	10.43	30.09
GR RC 03	X ± SD	18.87 ± 1.83	9.67 ± 0.80	9.92 ± 0.80	1.05 ± 0.23
	Variations limit	13.12 - 23.65	7.89 - 12.1	8.23 - 12.16	0.43 - 1.68
	CV %	9.71	8.32	8.13	22.32
ST RC 01	X ± SD	20.81 ± 2.42	12.01 ± 1.52	12.19 ± 1.55	1.63 ± 0.37
	Variations limit	13.87 - 25.14	1.70 - 15.91	1.77 - 15.98	0.43 - 2.88
	CV %	11.65	12.72	12.76	22.99
ST RC 02	X ± SD	19.59 ± 1.41	11.01 ± 0.71	11.10 ± 0.71	1.30 ± 0.25
	Variations limit	15.83 - 22.75	9.34 - 13.09	9.42 - 13.11	0.77 - 2.21
	CV %	7.24	6.48	6.47	19.28
ST RC 03	X ± SD	21.15 ± 2.16	11.56 ± 1.09	11.97 ± 1.11	1.43 ± 0.30
	Variations limit	14.51 - 26.10	8.36 - 13.98	9.38 - 15.16	0.72 - 2.29
	CV %	10.21	9.42	9.30	21.13
VL RC 01	X ± SD	18.38 ± 2.57	10.62 ± 1.15	11.07 ± 1.17	1.05 ± 0.32
	Variations limit	10.47 - 26.61	7.76 - 13.41	8.08 - 13.82	0.42 - 1.92
	CV %	13.98	10.85	10.62	30.87
VL RC 02	X ± SD	21.33 ± 2.42	12.34 ± 1.45	12.61 ± 1.44	1.60 ± 0.48
	Variations limit	15.95 - 27.68	9.24 - 16.14	9.41 - 16.25	0.75 - 3.25
	CV %	11.38	11.81	11.42	30.52
VL RC 03	X ± SD	19.81 ± 2.71	11.31 ± 1.09	11.61 ± 1.18	1.37 ± 0.34
	Variations limit	11.76 - 25.50	8.70 - 15.5	9.00 - 16.59	0.68 - 2.65
	CV %	13.70	9.68	10.19	25.22
All genotypes	X ± SD	20.02 ± 2.52	11.23 ± 1.40	11.50 ± 1.41	1.36 ± 0.44
	Variations limit	10.47 - 27.68	1.70 - 16.14	1.77 - 16.59	0.42 - 3.25
	CV %	12.61	12.47	12.33	32.86

Regarding the small diameter of fruit, the variations ranged from 1.70 mm (ST RC 01) to 16.14 mm (VL RC 02). The mean value was 11.23 mm, with limits ranging from 9.67 mm (GR RC 03) to 12.34 mm (VL RC 02). Roman et al. (2012), determined values between 8.7 and 14.2 mm for the average fruit diameter for rosehip genotypes from Transylvania.The observations made by Stoenescu & Cosmulescu (2021) show an average diameter value for all analysed genotypes of 13.00 mm. The coefficient of variation of small diameter for all genotypes is 12.47%, which shows uniform fruits for this characteristic. As for the large diameter, the data are approximately similar, the average value being 11.50 mm and the coefficient of variation having a calculated value of 12.33%. The variation limits for the mean diameter ranged from 9.92 mm (GR RC 03) to 12.61 mm (VL RC 02). For this characteristic, Benković-Lačić et al. (2022) specify average values between 13.07 and 13.28 mm for Croatian genotypes, and Tomljenović et al. (2021), between 11.36 and 15.17 mm. The weight of fruits has the widest limits, highlighted by the values of variability (19.23-30.87%), which can be explained by the fact that each genotype has a different maturation stage. The weight variation limits ranged from 0.42 g (VL RC 01) to 3.25 g (VL RC 02). The mean weight value of all genotypes analysed was 1.36 g. Regarding the coefficient of variation, the values were between 19.28% (ST RC 02) and 30.87% (VL RC 01), with an average value of 32.86%. A lower variability coefficient (18.90%) was calculated by Durul et al. (2023). These results also indicate a large variation in this trait with a notable influence of environmental factors, a fact also supported by Tomljenović et al. (2022).

Considering the recorded variability, a series of four histograms for each determined parameter was performed (Tables 3-6). Histograms visually interpret numerical data by indicating how many data points are within a range of values. The histogram is the most commonly used graph to show frequency distributions. Table 3 shows the grouping of fruits by fruit height. The height values were divided into 29 classes, but the maximum number of fruits (91) was in the range of values 20.22-20.80 mm, followed by the range 19.65-21.37 mm (83 fruits grouped into two classes); 28.56% of the analysed fruits have values between 20.22-21.37 mm, or 53.56% of fruits had values between 19.08-21.94 mm. The distribution of this histogram is uninomial, and the frequency curve appears asymmetrically positive.

Table 3. The fruit height histogram

Bin	*Freq*	*Cumulative %*	*Bin*	*Freq*	*Cumulative %*
20.80	91	10.11%	24.24	21	90.78%
20.22	83	19.33%	15.63	18	92.78%
21.37	83	28.56%	16.21	13	94.22%
19.65	79	37.33%	25.39	13	95.67%
19.08	77	45.89%	15.06	10	96.78%
21.94	69	53.56%	25.96	7	97.56%
18.50	61	60.33%	14.49	5	98.11%
22.52	56	66.56%	12.19	3	98.44%
17.93	42	71.22%	13.91	3	98.78%
16.78	37	75.33%	26.53	3	99.11%
23.09	36	79.33%	27.11	3	99.44%
17.35	35	83.22%	13.34	2	99.67%
23.66	24	85.89%	10.47	1	99.78%
24.81	23	88.44%	11.62	1	99.89%
			More	1	100.00%

Table 4 contains the histogram made for the parameter called the small diameter of the fruit and records a number of 19 classes, of which the one with the highest frequency is the class with limits of variation between 10.85-11.81mm (144 fruits; 16%). However, 88.89% of the values for large diameter are grouped into nine value points, as shown by the analysis of the data presented in Table 4. 64.33% of the analysed fruits had values between 10.36-12.29 mm and were divided into five classes of values.

Table 4. Histogram of the small diameter of the fruit

Bin	*Freq*	*Cumulative %*	*Bin*	*Freq*	*Cumulative %*
11.81	144	16.00%	14.21	15	94.11%
10.85	131	30.56%	8.44	14	95.67%
11.33	111	42.89%	8.92	14	97.22%
10.36	100	54.00%	14.70	12	98.56%
12.29	93	64.33%	15.18	4	99.00%
12.77	73	72.44%	15.66	3	99.33%
9.88	59	79.00%	More	3	99.67%
13.25	45	84.00%	7.96	2	99.89%
9.40	44	88.89%	1.70	1	100.00%
13.73	32	92.44%			

The histogram for the large diameter of the fruit is shown in Table 5.

Table 5. Histogram of the large diameter of the fruit

Bin	*Freq*	*Cumulative %*	*Bin*	*Freq*	*Cumulative %*
12.14	130	14.44%	9.18	16	95.11%
11.65	127	28.56%	14.61	14	96.67%
11.16	120	41.89%	8.69	11	97.89%
10.66	105	53.56%	15.60	6	98.56%
12.64	85	63.00%	15.11	5	99.11%
13.13	80	71.89%	16.10	4	99.56%
10.17	69	79.56%	More	2	99.78%
9.67	51	85.22%	1.77	1	99.89%
14.12	37	89.33%	8.19	1	100.00%
13.63	36	93.33%			

The values were grouped into 19 classes, but 89.33% were grouped into only nine classes. The highest frequency value was recorded in the class 11.65-12.14 mm (130 values, representing 14.4%). As in the previous case (small diameter), 63% of fruits were classified into five classes, with variation limits between 10.66-12.64 mm.

Table 6 refers to weight, representing the histogram for this parameter. The weight of the fruit is an important character of quality. The values obtained for this characteristic were divided into 30 classes of values, much more than for the diameter of the fruit. The maximum value reached by absolute frequency was 89 fruits for grades 1.27-1.36 g, representing 9.88%. However, even for this characteristic, 44.89% of fruits weighed between 0.99 and 1.55 g. Only 4.67% of values were contained in 13 classes.

Table 6. The fruit weight histogram

Bin	*Freq*	*Cumulative %*	*Bin*	*Freq*	*Cumulative %*
1.36	89	9.89%	2.02	16	92.67%
1.27	88	19.67%	2.21	16	94.44%
1.17	77	28.22%	2.31	8	95.33%
0.99	75	36.56%	2.50	8	96.22%
1.55	75	44.89%	2.59	6	96.89%
1.08	66	52.22%	0.61	5	97.44%
1.46	63	59.22%	2.40	4	97.89%
1.65	55	65.33%	2.68	4	98.33%
0.89	52	71.11%	2.87	4	98.78%
1.74	46	76.22%	2.78	3	99.11%
1.93	31	79.67%	2.97	3	99.44%
0.80	29	82.89%	0.51	2	99.67%
1.84	29	86.11%	0.42	1	99.78%
0.70	22	88.56%	3.16	1	99.89%
2.12	21	90.89%	More	1	100.00%

In order to establish the correlation between the two studied variables, the correlation (r) and determination (R^2) coefficients between the morphological characteristics of fruits in the analysed genotypes were calculated. The determined values are shown in Table 7. As can be seen for all calculated correlations, the value of r is between 0 and 1, which denotes that regardless of the pairs of grouped parameters, the values tend to increase or decrease together. As expected, the closest value to 1 is the correlation between the two diameters (r = 0.979). There are high values for both the weight-height correlation of the fruit (r = 0.717) and the weight - diameter of the fruit (r = 0.830).

The coefficient of determination (R^2) is a statistical measurement that examines how the difference in a second variable can explain differences in one variable. From the data obtained (Table 7) it appears that between 54.15 and 73.65% of the weight of the fruit is predicted by the dimensions of the fruit (height and diameters). Only 24.89% of the large diameter variable and 26.86% of the small diameter influence the height of the fruit, which indicates that the shape of the fruit is a genetic character characteristic of each genotype.

Table 7. The correlations of the studied parameters

*	Indices	H (mm)	d (mm)	D (mm)
H (mm)	R^2	1		
	r	1		
d (mm)	R^2	0.2686	1	
	r	0.556	1	
D (mm)	R^2	0.2498	0.9587	1
	r	0.543	0.979	1
G (g)	R^2	0.5415	0.7365	0.7312
	r	0.717	0.830	0.830
*H= Fruit height; d= Small fruit diameter; D= Large fruit diameter; G= Fruit weight				

The value of 0.9587 of the coefficient of determination between the two diameters suggests that 95.87% of the dependent variable (D) is predicted by the independent variable (d).

CONCLUSIONS

Although the studied areas are quite close geographically, the differences between genotypes are significant, with a rather large variability of *Rosa canina* L. in the southern area of Olt County, especially regarding fruit weight. The limits of variation are vast, both within and between genotypes, including increased variability. Comparing with the above-mentioned literature, it can be accepted that the fruits studied are of medium size but may constitute biological material for both research and recovery.

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COMPARISON APPLE BIO PRODUCTION ON CULTIVATED AND GRASSED AREA

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Abstract

Biological agriculture is an important priority in the thematic priorities of the European scientific programs and in agricultural development policy in each country. This article presented apple bio production in the territory of the village of Yagodovo, Plovdiv district, Bulgaria. Growth parameters are monitored on two types of areas - cultivated and grassed. The analysed indicators are cross-sectional area of the stem, average weight of fruit, number of fruits per tree, productivity coefficient. Each of the parameters has a different impact on the biological development of the fruits. The results show better values when growing on a grassed area compared to a cultivated area, regarding the parameters: average weight of fruit, number of fruits per tree and productivity coefficient. The indicator `cross-sectional area of the stem` has a better result when growing apples on a cultivated area than on a grassy area. The results for each of the indicators are presented in a digital model by Geographic Information System-GIS, in order to more fast, rational and modern use of technology by young farmers.

***Key words**: apple growing parameters, organic production, cultivated area, grass plot area, GIS.*

INTRODUCTION

Organic fruit production is a system that limits the use of artificial fertilizers, synthetic pesticides, growth regulators and genetically modified organisms (Ordinance No. 1 of 2013). The key principles and practices of organic production aim to maintain long-term soil fertility, supply plants with the necessary nutrients through natural or organic fertilizers, control weeds, enemies and diseases through crop rotation, use of natural predators and limitation of chemical treatments in order to protect the environment- biodiversity, water, etc. (Peck et al., 2006; Herencia et al., 2007; Merwin and Peck, 2009; Singhet al., 2009).

In recent years, the areas for organic production have been increasing, but for the venture to be successful, it needs effective management of soil fertility, ensuring economic profit and maintaining fruit quality standards.

The market for organically produced fruits grows and attracts more and more customers who choose a product with desired qualities and the idea that organic fruit production is a promising economic model is strengthened. Europe is a major global market and producer of organic food and the area continues to grow.

In Europe and in many countries around the world, the demand and production of organic fruit is increasing. Fresh fruits and vegetables are the most preferred organic products in Europe and are about 1/3 and 1/5 of those sold in markets.

Europe is the world leader in the sale and production of organic food. Areas under fruit crops include 192,700 ha of vineyards, 187,000 of nut, 94,800 of temperate fruit, 26,096 of berry and 31,800 of citrus. Western Europe has added a new 4,000 ha of organic apple production in the last two years, due to the increased market demand for organic fruit and the reduction in the cost of conventional production (Staneva and Gospodinova, 2018).

The development of apple industry played an important role in increasing the income of farmers and improving the live style people. However, in Bulgaria, the increasing in apple production is mainly dependent on the high input of agricultural means of production, particularly chemical fertilizers and pesticides. Statistics showed that Bulgarian's apple cultivation area accounts for 4433 ha, whereas the consumption of chemical fertilizers and pesticides increase. The excessive use of fertilizers and pesticides not only leads to the waste of resources, but also brings water eutrophication, increases greenhouse gas emissions, and further degrades soil quality (Guo et al., 2010; Zhang et al., 2012), which, in turn, are all crucially connected to food safety problems, thereby prompting extensive social discussion on how to resolve these issues.

In Bulgaria, there are prerequisites for organic fruit production, but a lot of knowledge and a great desire of the producers are needed to deal with this type of production. In the country, organically produced fruit species are grown on 2155 ha.

With the intake of healthy foods, one of the positive aspects of organic farming is the desire to reduce the harmful effects of the application of pesticides and mineral fertilizers, since organic farming restores the natural balance in the environment.

The yields in organic production of apples are often 15-30% lower in comparison with conventional or integrated production guidance. Inconstant and lower yields are often due to insufficient fruit load, ineffective control of enemies, diseases, weeds and nutrient deficiencies. The reasons are: lack of sufficiently effective plant protection from diseases and enemies, strong competition with weeds, distinct alternative fruiting (Weibel, 2002; Bertschinger et al., 2004; Gianessi and Williams, 2006).

The studies by Reganold et al. (2001) of conventional, integrated and organic apple production in the USA showed that there were no significant differences in yields, but the organic system provided better soil quality and the environmental impact was lower compared to the conventional system. When comparing conventional and integrated systems with organic, it turns out that the latter provides sweeter and less acidic fruits, higher yield and greater energy efficiency.

A similar study was conducted in southern Brasilia, where yield and quality were assessed of fruit production in two varieties of apples (Royal Gala and Fuji) under conventional and organic production systems (Amarante et al., 2008). Trees grown under the biological system had lower potassium, magnesium and nitrogen content in leaves and fruit in the cultivars, smaller fruit and lower Fuji yield compared to the conventional system. Fruits picked when ripe for consumption from the organic garden have a stronger yellow skin colour, a higher content of soluble salts, density, hardness, more strong reddish-brown coloration.

The already established intensification in agriculture necessitated the use of non-traditional products in applied agricultural techniques for growing different crops. One of them is a soil moisture superabsorbent. As a result of its use, mainly in the production of fruit beds and in vegetable production, high achievements have been registered in terms of yields, savings of the most valuable natural resource - water, savings of human labour, improvement of soil structure, microbiological activity, good development, both on the above-ground mass of plants and on their root system, etc. (Popova et al., 2016; Dobrevska and Dallev, 2020; Atanasov et al., 2020).

These are the reasons why this product was studied in horticulture in the production of apple fruits. The aim was to determine to what extent changes in soil components, as a result of using a moisture absorbent, will change growing conditions and lead to obtaining first-quality apple fruits.

MATERIALS AND METHODS

This study presented information about previously applied agricultural practices in the field of apple bio cultivation. The presented practices have been managed for a period of five years in an orchard with Florina variety – grafted on MM106 rootstock – as a part of bio production of fruit at the Agroecological Centre of the Agricultural University - Plovdiv.

The experiment was conducted in experimental apple plantations of the Florina variety at the Agricultural University - Plovdiv, grown in a organic way.

The plants in the plantation for organic production are grown according to traditional technology. It includes plant protection measures (three prophylactic winter ones, directed against the wintering forms of economically important diseases and enemies and numerous vegetation ones), nourishing fertilizing, localized drip irrigation and several soil treatments. The processing was done with a specialized technique for deep and shallow processing in fruit plantations (Borovinova and Juvinov, 2013; Todorov et al., 1974). The inter-row distances in the plantation are maintained

in two ways – through a fallow system (cultivated area) and a mulching system (grassed).
In connection with the first, during the growing season, several shallow treatments were made in the inter-rows, which would lead to a good supply of nutrients, a good air and water regime. The treatments are also used to destroy weeds.
In autumn, a deep processing of the rows at 18-20 cm was also done. The tillage machinery system used is: plough, disc harrow, cultivator and tiller. In the second system of maintaining the rows, they are grassed, and the grass is periodically mowed.
Mowing is done with a mulcher, which saturates the mowed mass and places it in the inter row on the grasses. With their roots, they contribute to the good loosening of the soil, which leads to a favourable air and water regime in it. In this connection, the microbiological activity is also improved and the soil is enriched with organic substances.
The other agricultural practices carried out in the plantations are fertilization and plant protection. Fertilization was carried out with a centrifugal mounted fertilizer, equipped with deflectors for applying the granular fertilizers close to the trees. With a trailed fan sprayer, in addition to plant protection, liquid fertilizers were also applied.
The plants in the plantation for biological production are grown using technology without the application of mineral fertilizers and synthetic pesticides.
Bio fertilizers and bio plant protection preparations were used. The inter-row soil surface is supported by the same two systems described above.
Irrigation is well dosed and localized drip. In the spring, during the "early growth" phase of the "growth" phenophase, moisture absorbent was applied to the soil at a dose of 5.5 kg/dka.

RESULTS AND DISCUSSIONS

In this study, information regarding previously applied agricultural practices in the field of apple cultivation is presented to your attention. The practices have been conducted for a period of five years with Florina apple variety orchards, grafted on MM106 rootstock, as a part of the bioproduction process at the Agroecological Center of the Agricultural University - Plovdiv, located nearby the village of Yagodovo, Plovdiv region.

Figure 1. Llocalization of the researched area within the borders of the Republic of Bulgaria

For maximum results when growing crops in an organic production environment, it is good to apply modern methods of localization and good presentation of the results of the experiments through the so-called Geographical Information System (GIS). The possibilities of GIS for presentation and analysis of the results are many and different, but the most important role of the system is the presentation of research visually on maps. In this way, the results are easier to perceive, the analyzes of multiple data are fast and easy to edit and modify. Multi-level information is created that is easy to transform into maps showing the results achieved.
In the study of different growth and reproductive indicators of apples variety Florina through bioproduction on a cultivated and grassed area, GIS presents the studied territory and its localization.
The village of Yagodovo is part of the Rodopi Municipality, which is located in southern Bulgaria, southwest of the city of Plovdiv and administratively belongs to the Plovdiv region of the same name. According to the provisions and territorial division of the Regional Development Act, it is included in the South Central Planning Region (South Central Planning Region, NUTS 2).

Figure 2. Map of the Yagodovo village in Plovdiv region

The village of Yagodovo is located in the Thracian plain, 9.5 km from the center of Plovdiv. It is located at 165m above sea level in the southern part of Bulgaria. The terrain is mostly flat with gently sloping terrain. For the studied territory, the average annual temperature is about 18°C.

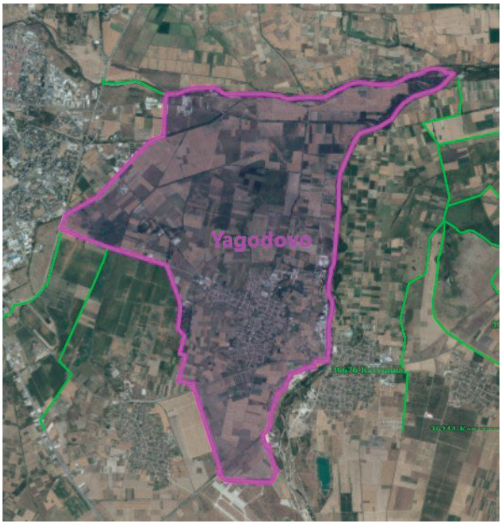

Figure 3. Terrain map of the studied area

Average maximum temperatures are within 30°C and average minimum temperatures are around 6.5°C. The amount and distribution of precipitation during the year varies widely, with a tendency to regroup in one spring and one winter maximum, within about 530-560mm. The plain territories of the Rodopi municipality are of the alluvial - deluvial meadow type, deluvial and deluvial-meadow type, with a predominant representation of the deluvial ideluvial-meadow soils.

The combination of very good soils, with a temperature sum for the growing season of 4,400°C, allows even the most heat-loving crops to be grown on these grounds. The low water reserves in the flat areas and the good soil and climate conditions for the development of an efficient agricultural sector, determine the need to introduce irrigation into the agricultural practice agriculture and technology (Integrated Development Plan of мunicipality of Rodopi for the period 2021-2027). The next map presented the studied area in coordinated systems WGS 84 / UTM zone 35N, the urban and agriculture parcels.

Figure 4. Property boundaries of the agricultural territory of the studied area

As a result of the experiment, higher values of instantaneous humidity were recorded during one of the most significant phases of the phenophase of apple growth - the "strong growth" phase. During the previous growing season, seven irrigations were made in the experimental plots by means of a drip system and an irrigation rate of 40 m^3/dka. As a result of the experiment, watering was reduced to four. A higher productivity coefficient was also reported (Table 1). It expresses the weight of the fruit relative to the cross-sectional area of the stem. In both variants, with cultivated and cultivated area, higher values were shown by the trees in the cultivated areas.

Table 1. Growth and reproductive manifestations of apples variety Florina in organic production

Florina apple bio production		
	Types of area	
Parameters	Cultivated	Grassed
Cross-sectional area of the stem (cm^2)	116,92	110,05
Average weight of fruit (kg)	0,151	0,172
Number of fruits per tree	116,15	121,57
Productivity coefficient (kg/cm^2)	0,15	0,19

The plants from biological production field were cultivated according to a technology excluding the use of mineral fertilizers and synthetic pesticides. Only bio-fertilizers, as well as plant-protection agents were used. The soil surface in the inter-row space was maintained according to the aforementioned two systems. Localized drip irrigation was precisely-dosed.
During the research, growth and reproductive indicators of apples of the Florina variety were considered. The territory of the village of Yagodovo is favorable for the cultivation of organic production. The apple orchards are grown on two types of land - cultivated and grassed. The indicators that have been studied are: cross-sectional area of the stem (cm^2); mean fruit mass (kg); number of fruits per tree; productivity factor (kg/cm^2). In both variants of the area, the same plants were studied. The investigated indicators give different values for different areas.
In apple plants grown on a cultivated area, the index of cross-sectional area of the stem has a better value of -116.92 (Table 1), compared to apples grown on a grassy area with a coefficient of 110.05.
The indicator number of fruits per tree has similar values, with the harvested area having about 6 more apples than the cultivated area. The average weight of a fruit has better indicators in a grassy area than in a cultivated one.
The indicators are the same when studying the coefficient of productivity, and again with a grassy area the values are better.
In general, three of the studied indicators have better values when growing apples in a grassy area. This leads to the conclusion that in a grassy area apples of the Florina variety have better growth indicators.
Organic apple production on grassed area shows much better fruit parameters and leads to better economic and market benefits.
The main concern of organic production is that all activities protect the environment. At the same time, they must be economically viable. The implementation of plant protection measures according to these requirements is changing. It is economically acceptable for crops to be effectively protected against disease and pests, taking into account the impact on organisms.
This information, combined with future studies of soil composition, as well as vegetative and reproductive manifestations of plants, can be used to develop a database enhancing the access to knowledge and experience in the future and facilitating the comparison and selection of the most appropriate agricultural practices for apple cultivation.

CONCLUSIONS

A number of ecological factors influence fruit preservability and quality. While it is difficult to assess the weight of each factor individually, either one of them, including soil type, latitude, altitude and temperature fluctuations, etc., may be of significant importance.
The studied system for the production of apple fruits in the experimental plots for organic production with cultivated and weeded inter rows development shows:

- Reduction of watering during the vegetation;
- Higher values of instantaneous humidity during one of the most important phases of tree growth;
- Higher productivity factor.

This system can be implemented in practice and used as a model system in the production of fruit and other fruit species.

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CHANGES IN PHENOLOGY OF SERBIAN PLUM VARIETIES UNDER AGROECOLOGICAL CONDITIONS OF THE CENTRAL BLAKAN MOUNTAIN REGION IN BULGARIA

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Abstract

For the period 2019-2022, the phenological manifestations were studied of the Serbian plum cultivars such as 'Čačanska rana', 'Čačanska najbolja', 'Čačanska lepotica', 'Čačanska rodna', and 'Valjevka' and their response to climatic factors such as temperature, rainfall, relative air humidity in RIMSA-Troyan. The trees are grown on light gray forest soil, under non-irrigated conditions, with tillage between the inter-row spacing. The phenophases were studied, such as the beginning of flowering, end of flowering, and fruit ripening periods. The earliest flowering began in 2019 for 'Čačanska rodna' cultivar (28.03.), and the latest flowering began on 6.04.2019 for 'Kyustendilska plum'. In the following years, spring temperatures delayed the vegetation onset and the beginning of flowering was around 07.04 for 'Čačanska lepotica', followed by 'Čačanska rodna' (10.04.) and 'Čačanska najbolja' (12.04). The latest end of flowering was reported on 7-8 May 2022 ('Valjevka' and 'Kyustendilska plum'). For the entire study period, the phase from flowering to harvest maturity was shortened. To obtain favorable conditions for growth and fruit-bearing in the Central Balkan Mountain region, the introduced Serbian cultivars require changes in cultivation technology and adaptation to changes in the global climate.

***Key words**: climatic factors, phenology, plum cultivars.*

INTRODUCTION

Prunus domestica L. is one of the most frequently grown fruit growing species in Bulgaria because of its high ecological adaptation and the various directions for the use of the fruits. In 2022, plums and cherry plums had the largest share of the entire fruit production, followed by cherries and apples, with a relative share of 25%, 24%, and 21% respectively (https://www.mzh.government.bg/bg/statistika-i-analizi/izsledvane-rastenievadstvo/danni/).
'Stanley' variety is widely grown in orchards and, according to Agrostatistics Department, it occupied 73.4% of orchards in Bulgaria in 2019.
According to FAOSTAT (2022), Serbia ranks among the largest producers of plums in the world, taking third place after China and Romania, according to the production in 2022 (488,593 tons). As the development of the market is focused on the quality of fresh plum fruits, in recent years there has been a growing demand for fruits of larger size and good quality for fresh consumption (Tomić, 2022).
There is a need to expand the assortment and introduce plum varieties tolerant to *Plum pox virus,* with good economic qualities and different ripening periods, suitable both for fresh consumption and processing.
The monovarietal structure consisting of the 'Stanley' variety can be changed by introducing new plum genetic resources and creating new varieties that meet the latest requirements of producers and consumers (Bozhkova, 2013). Successful plum production requires well-adapted varieties to the specific growing conditions of the production area. Due to the interaction between the environment and the genotype, it is very important to evaluate in advance the agro-ecological conditions of the area and the pomological characteristics of the variety.
The leading place of autochthonous varieties in the structure of varieties is decreasing. It is slowly being taken by varieties with combined characteristics, such as 'Cačanska lepotica' and 'Čačanska rodna', as well as 'Stanley', which, despite its numerous shortcomings, is one of the

leading varieties in Serbian plantations (Tomić et al., 2019).
'Čačanska lepotica', 'Čačanska rodna', 'Čačanska najbolja', 'Valjevka', were introduced at the Research Institute for Fruit Growing of Pitesti in Romania (Butac, 2021). In 2018-2020, flowering and ripening periods, yield (kg/tree^{-1}), growth vigor, and susceptibility to *Plum pox virus* (PPV) were studied. For the Pitesti region, the flowering of the investigated cultivars took place in the first half of April, and the average ripening period varied from the beginning of July ('Boranka') to the end of August ('Mildora' and 'Čačanska Rodna'). 'Čačanska Lepotica', 'Čačanska Najbolja', 'Mildora', and 'Valerija' are reported as high-yielding varieties (over 15 kg tree^{-1} in the 5th year after planting). Most of the introduced Serbian varieties showed very good results in Romanian climatic conditions, but the most common was 'Čačanska Lepotica'.

Szabó & Nyéki, (1996) studied 63 European plum varieties on 6 sites of different specifics between 1982 and 1989. It was found that the development and duration of flowering were mainly influenced by air temperature. An average daily temperature below 10°C results in a stigma viability period of 3 to 5 days versus 1 to 2 days when it is above 13°C. Anthers open slowly below 14°C and rapidly above 20°C. Plum varieties are divided into 3 groups according to the duration of flowering: short (less than 8 days), medium (8 to 11 days) and long (more than 11 days). According to the period of flowering, 5 groups were formed: early, medium-early, medium, medium-late, and late. The flowering periods of varieties in the same group or adjacent groups overlap to a large extent.

Phenological changes are one of the most striking manifestations of global climate warming. The extent to which plants are affected by changes in temperature and rainfall, their inherent adaptive capacity determines their production potential, the ecological stability of ecosystems, and food security (Cosmulescu, 2023)

In general, it is observed that the main response to climate change is expressed in the length of the vegetation period, the earlier onset of spring phenophases, and the delay of autumn ones. The impact of long-term climate change on cherry and apple development was identified as differences in flowering period and compared in ten regions of Central Europe. Early flowering was found in all regions for the period 1951-1995 (Roetzer et al., 2000). When investigating the impact of environmental factors on the phenology of fruit orchard species, decisions can be made regarding the location of varieties in different growing areas, depending on local environmental conditions (Cosmulescu et al., 2008). The analysis of the species response to the climate shows a linear dependence (correlations) of the phenophases with the early spring climatic conditions. A warming trend was also found, especially for November and January (Cosmulescu, 2023).

The duration of the flowering period is a characteristic that is influenced by climatic and genetic factors. The impact of the meteorological factor manifests itself in different years, thus causing in the same variety a different duration of time between the beginning and end of flowering. In general, the earlier flowering develops, the shorter its duration (Cosmulescu et al., 2010).

Chmielewski et al. 2003 showed that a 1°C increase in average air temperature between February and April caused an early start of vegetation and flowering of fruit trees by about 5 days.

The objective of the present study is to show a phenological calendar for 2019-2022 and the behavior of Serbian varieties to the changes in the climatic conditions of the Central Balkan Mountain region in Bulgaria.

MATERIALS AND METHODS

The study was conducted in the period 2019-2022 on the territory of RIMSA in Troyan (42°53′N 24°43′E, altitude 420 m). The plum varieties, such as 'Čačanska rana', 'Čačanska najbolja', 'Čačanska lepotica', 'Čačanska rodna', 'Valjevka', 'Stanley', 'Kyustendilska' plum were studied.

The trees are grafted on a cherry plum rootstock, in a period of full fruit bearing. They are grown on light gray forest soil, under non-irrigated conditions, with tillage between the inter-row spacing, at a planting scheme of 5 x 4 m.

The phenological manifestations were studied by monitoring the climatic factors such as temperature, rainfall, and relative air humidity, based on data from a stationary meteorological station on the territory of RIMSA.

Flowering phenophase was studied by observing and recording the flowering onset (10% open flowers), full bloom (80% open flowers), and end of flowering (90% petal fall) and determined according to Wertheim (1996). The period of physiological maturity is determined based on the number of days from full flowering to harvest.

The tree yield (kg) was analyzed. The standard error section was added as a statistical data processing, and regression equations were derived, using Analysis Tools Microsoft Excel.

RESULTS AND DISCUSSIONS

Climate

Average daily temperatures at the beginning of March were extremely low in 2022 (0.5°C). It was higher in 2019 (8.0°C) and 2020 (8.9°C). For each subsequent year in 2021 (3.6°C) and in 2022 it became lower (0.5°C) (Table 1). In 2019 and 2020, the average monthly temperatures was about 6.8-7.8°C, but in 2021 it was 3.3-3.9°C, and in 2022 it was from 0.5°C at the beginning of the month to 8.7°C at the end. April for the first 2 years was cooler, and in the second ten-day period temperatures reached 8-11°C, but in 2021 it gradually increase from 6.3 to 7.3 °C and at the end of April it reached 11.3°C, which did not repeat in 2022. Then high temperatures were observed at the beginning of April (11.1°C), with a decrease to 7.2°C in the middle of the month and warming to 13.5°C at the end of April (Table 1).

In 2022, the average monthly temperature in March was 3.2 (°C), and in February was 3.8 (°C). This difference completely determines the changes that affected the phenological development in 2022. The cooler temperature in March (from 8.03 to 13.03, and on 19.03 and 20.03 with negative daily mean values) delayed the flowering processes.

The smallest amounts of rainfall were reported in May (28.8 mm) and July (35.0 mm). The total amount of rainfall for the 2022 vegetation was 361.6 mm (Figure 1). For comparison, the vegetation amount of rainfall in 2021 was 285.4 mm, as this was the lowest recorded vegetation rainfall amount for the last twenty years.

In 2020 and 2021, there was evenly distributed rainfall for each ten days of the spring months. It was about 10 mm in 2020, and about 20 mm in 2021. In 2022, at the beginning of March with 0.5°C, there were 15.4 mm, at the end of the month there was no rainfall, and in April the total amount was 50 mm.

All these factors have an impact on the phenophases of plum varieties in the conditions of the Troyan region.

Table 1. Climatic factors for 10-day periods in March and April

	March			April		
2019	**1-10**	**10-20**	**21-31**	**1-10**	**10-20**	**21-30**
Relative humidity (%)	61.0	69.0	62.0	68.0	79.0	70.0
Rainfall (mm)	1.6	14.3	0.6	50.4	45.1	11.4
Temperature (°C)	8.0	7.9	6.8	9.8	8.1	12.0
2020						
Relative humidity (%)	72.9	69.2	77.6	70.7	66.4	69.0
Rainfall (mm)	39.0	5.6	8.8	10.6	9.2	4.6
Temperature (°C)	8.9	7.2	5.3	5.9	11.3	11.1
2021						
Relative humidity (%)	69.1	83.1	75.2	72.7	75.1	73.3
Rainfall (mm)	5.1	20.0	22.6	26.0	20.6	10.4
Temperature (°C)	3.6	3.3	3.9	6.3	7.3	11.3
2022						
Relative humidity (%)	82.5	68.4	56.5	66.3	60.1	76.6
Rainfall (mm)	15.4	7.0	0.0	45.8	40.4	9.6
Temperature (°C)	0.5	-0.1	8.7	11.1	7.2	13.5

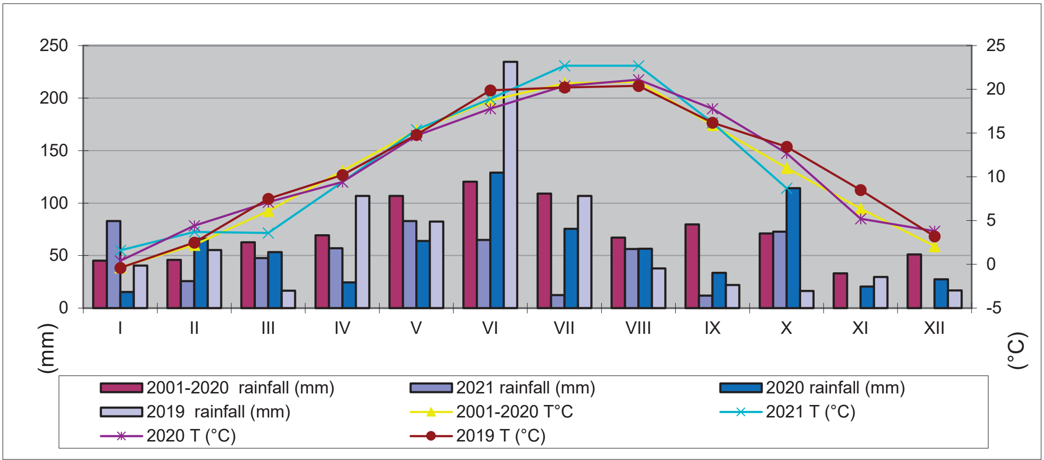

Figure 1. Average monthly temperatures and total rainfall amounts (2019-2022) and 30-year base period (1988-2017)

Changes in climatic factors are in the direction of global warming. In the period January - May, the changes in the course of temperatures and during the phases vegetation onset were provoked, but then the development of the plant stopped and the flowering phenophase was delayed.

The earliest flowering began in 2019, as the average temperatures of 10-12°C in the first ten days of April (Table 1) caused the beginning of flowering. The earliest flowering began in 2019 for 'Čačanska rodna' variety (28.03.) on the 88th day, followed by 'Čačanska lepotica' on the 89th day, as the latest flowering began for 'Kyustendilska plum' on 6.04.2019 (96th day) (Figure 2).

In the following two years, the spring temperatures in the third ten days of March and the first ten days of April were below 6°C, which delayed the beginning of flowering compared to 2019. In 2020, the beginning was reported on 07.04 for 'Čačanska lepotica' (98th day), followed by 'Čačanska rodna' (100th day) and 'Čačanska rana' (101th day).

In 2021, the beginning of flowering was delayed by the low temperatures (from 4 to 11°C), but flowering started even later in 2022 because in March there were negative temperature values and abundant rainfall amount (40-45 mm). A favorable period for the beginning of flowering was the last ten days of April when temperatures permanently exceeded 10°C (Table 1).

In general, for the period of the study, the trend of a gradual delay of the beginning of flowering is outlined. 'Čačanska lepotica', which is the earliest flowering variety, began flowering on the 89th calendar day in 2019, in the following years on the 98th; 105th; and 115th, the difference from the first to the last year is 17 days. The 'Stanley' variety began its flowering phase on the 94th day in 2019, respectively 102nd day (2020); 104th day (2021), and 115th day (2022), with a delay of 21 days.

The duration of the full bloom phase (80% open flowers) was from 10 to 18 days in 2019 and 2021, as it was shorter in 2020 and 2022 (10 to 15 days). Milatovic et al. (2018) determined that the average duration of flowering for all varieties was from 7.8 days in 2015 to 11.5 days in 2016, as the most abundant flowering was reported for the 'Valerija' variety (as well as for the control 'Čačanska lepotica').

The moment of overlapping of full blossoming of the 7 varieties in the present study is 6 to 10 days, in all years, which gives us reason to recommend using them in the same plantation, to favor cross-pollination.

The latest end of the flowering period was reported on 7-8 May 2022 ('Valjevka' and 'Kyustendilska plum' varieties).

The annual delay of the onset of flowering reduces the risks of late spring frosts in the area.

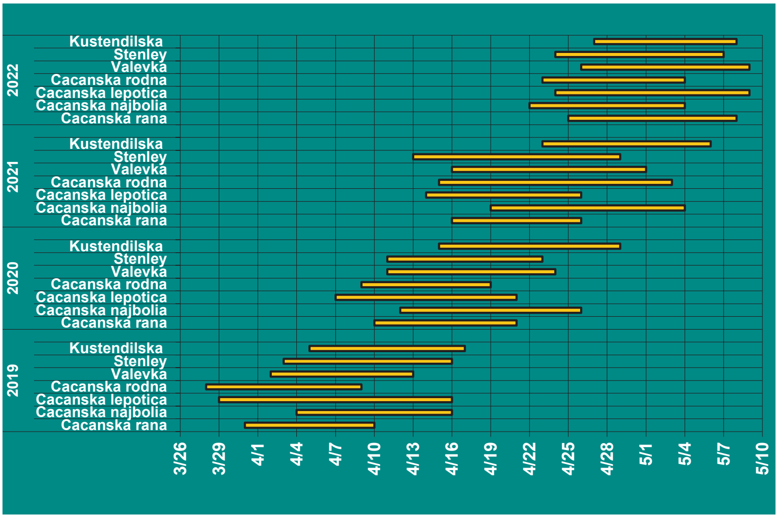

Figure 2. Flowering phenology of plum varieties

The sequence of the ripening period is the same, as a genetic trait of the varieties, but the annual global warming of the climate accelerates the onset of the ripening period.

'Čačanska rana' variety ripened first on July 15/20, followed by 'Čačanska lepotica' (August 5/6/10) in each subsequent year (Figure 3).

In 2019, the earliest ripening process was observed on the 199th day for 'Čačanska rana', and in 2020 on the 197th day. In the next 2 years, it was delayed and the variety ripened on the 201st day (2021) and the 203rd day (2022).

'Čačanska lepotica', in the first and second years, reached harvest maturity on the 217th/219th day, and in the following 2 years on the 222nd day. This indicates an approach to the ripening deadline and a shortening of the phenophase period from flowering to ripening over the years due to climate change, to higher summer temperatures and more severe droughts in the summer months, during the period of fruit ripening.

The latest ripening variety is the 'Kyustendilska plum'. An extremely significant shortening of the period of harvest maturity was observed. Except for the second year, its fruits ripened on 13.09 in 2019 (256th day), on 12.09. 2021 (255th day) and on 8.09.2022 (251st day) (Figure 3).

Because of the drought in late summer and autumn, trees do not normally stock up with nutrients for the period of forced dormancy, and accordingly, in each subsequent year, harvesting will require higher agrotechnical care and technological security, such as tillage, feeding, and irrigation.

Premature fruit ripening is a factor in alternation in fruit bearing. High autumn temperature and lack of rainfall (Figure1) slowed down and almost stopped the outflow of nutrients, plant storage was weaker and led to exhaustion, and the risk of frost in winter was a prerequisite for the alternation in fruit-bearing, since the differentiation of fruit buds (15.08-15.09) could not flow in the right direction.

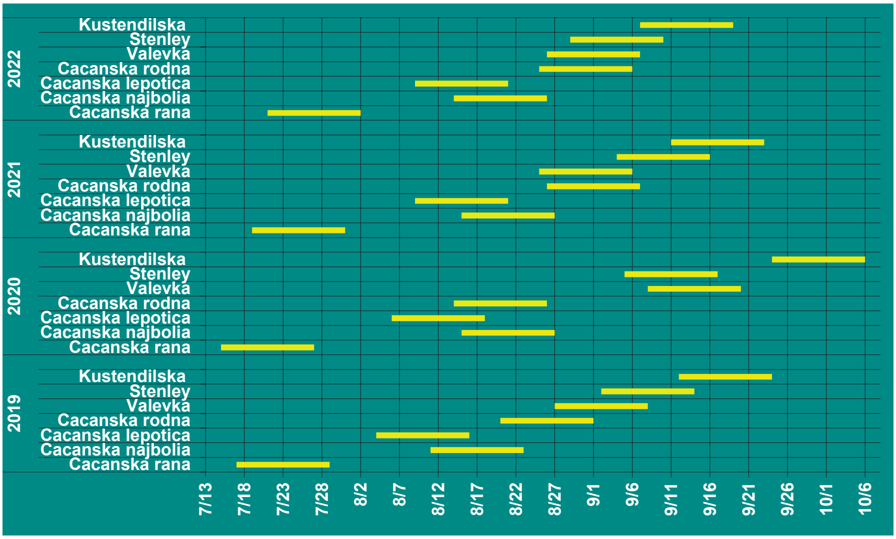

Figure 3. Ripening period of plum varieties

The measures that can compensate for these negatives to respond to the physiology of the tree are watering, and nutrient supply (more often with smaller doses of fertilizers), but this requires changes in cultivation technologies in mountain fruit growing. In the previous practice, irrigation was not applied, but in order to ensure fruit production with regular and high yields of quality fruit, it will be necessary. And on the other hand, changes in market requirements will also require an intensification of production technologies.

The difference between years with the earliest and latest fruit ripening according to Milatovic et al. (2018), varies from 7 to 15 days. The flowering and ripening periods of plum varieties in the Belgrade region is earlier compared to the Czech Republic, Central Bulgaria, and Northern Montenegro. These differences can be explained by the different environmental conditions between the studied regions.

Yields are directly dependent on the normal course of the phenological phases of the plants and the appropriate climatic conditions for fruit size and ripening (Figure 1 annual climate). In 2019, yields varied from 20 to 40 kg, according to the genetic capabilities of the varieties. In 2020, Serbian varieties showed significantly higher yields, compared to the previous year, whereas Stanley and 'Kyustendilska plum' gave lower yields (Figure 4).

In the extremely dry 2021, the lowest yields were reported for 'Čačanska rana' and 'Čačanska najbolja' (4-10 kg), which are early ripening. 'Stanley', 'Kyustendilska plum', and 'Čačanska lepotica' had yields of 26-34 kg per tree, as a result of the higher yields than in previous years and droughts, the depletion of trees led to a sharp drop in yields (Figure 4.)

In 2022, harvests were not reported. The reason for this is that after the abundant full blossoming at the end of April, the minimum temperatures at the beginning of May (2/4.05.) fell to 1.7-3.2°C (Figure 1). They were measured in the meteorological station on the territory of RIMSA, but in the orchard and the specific location of the area they were from 0°C to -3°C. Their influence lasted for more than an hour (between 5 and 6 in the morning), which in the initial phase of the development of the fruit-set (petal fall - formed fruit-set) caused the freezing of a large part of the fruit organs and the fruit yield was completely compromised.

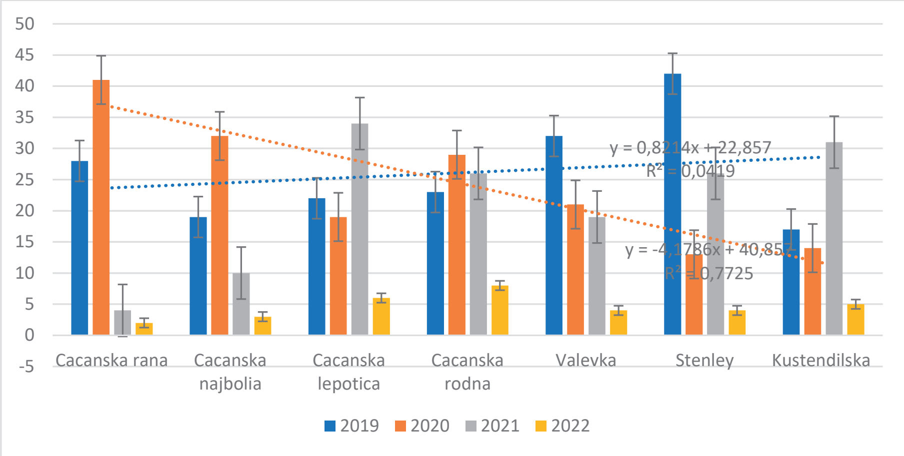

Figure 4. Fruit yield (kg/tree)

If the optimal agrotechnical events and plant protection are, the average optimal yields per variety would be 60-80 kg per tree.

Figure 4 shows that in 2019 the yield trend line corresponds to the linear regression y=0.6025x+22.857, with a coefficient of determination R^2 = 0.0419, i.e. the variance of the outcome variable (yield) is dependent on the action of the factor variable (variety) by 4.2%. In 2020, with the equation y = (-4.2236) x+40.857, R^2 = 0.7704, the influence of the variety on the yield is 77.04%.

CONCLUSIONS

Climatic changes are in the direction of warmer winters, which causes an earlier vegetation onset, but the flowering phases were delayed due to cold weather in the spring months.

For the entire study period, it was found that the phase from flowering to harvest maturity was shortened, due to an increase in the average temperatures in July, August, and September. Early ripening of the fruits was provoked and the end of the vegetation occurred more quickly.

The studied varieties are suitable to create a plantation, according to the flowering periods, since the phenophase overlaps from 6 to 10 days in all of them.

To obtain favorable conditions for growth and fruit-bearing in the Central Balkan Mountain region, the introduced Serbian varieties require changes in cultivation technology and adaptation to changes in the global climate.

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FRUIT QUALITY OF APPLE VARIETIES CULTIVATED IN AN ORGANIC SYSTEM

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Abstract

Apple is an important crop in many European countries. There is a growing interest, from consumers, for fruits obtained through ecological cultivation techniques. In order to satisfy this demand, some products compatible with ecological agriculture were tested applied to the soil and foliage of an apple crop in the Arges Meadow. The aim of this study was to evaluate the impact of organic fertilizers on production quality. Larger fruits were obtained from the organic treatments. The results also indicate a surplus of malic acid, sugars and total phenols in all varieties compared to those in the control variant. We conclude that through radicular and extraradicular fertilization, the apple varieties 'Romus 3', 'Idared' and 'Golden Delicious' have a higher nutritional quality.

Key words: *apple, organic fertilization, fruit quality, biochemical characteristics.*

INTRODUCTION

The apple (*Malus domestica* Borkh.) is one of the most important fruit crops in many countries (Cornille et al., 2012), including in Romania, and their quality is very important in consumer acceptance. In 2022, the apple occupied the third place as the most consumed fruit, worldwide (Dodocioiu & Buzatu, 2023).

Fertilizer management systems used in apple cultivation worldwide correspond to organic farming (only use of organic fertilizers), natural conditions (no fertilizers or pesticides) and the use of organic fertilizers combined with chemical ones (Kai & Kubo, 2020; Peck et al., 2006). In order to reduce the risks to health and the environment, new methods are being tried to reduce the administration of pesticides and chemical fertilizers. Organic cropping systems exclude the use of chemical fertilizers and pesticides, allowing the use of manure, compost, copper compounds, organic insecticides, pheromonal traps and other means of biological control (Holb et al., 2003; Peck et al., 2006; Jönsson, 2007). Biopreparations are fertilizers that contain one or more organic compounds such as enzymes, plant hormones, amino acids or vitamins. Bio-preparations can also contain macro or micro elements (Tarozzi et al., 2008). Although at the national level there have been major increases in organic agricultural commodities (25-50% per year) (Oltramari et al., 2002 cited by Amarante et al., 2008), the production of apples grown in organic systems in Romania is incipient.

If until the middle of the 19th century, the only way to provide plant nutrients at the soil level consisted in the application of manure (Zuoping et al., 2014), at the moment culture technologies use a wide range of products. According to Xu et al. (2008) administration of organic manure improves soil quality. Edmeades (2003) reported lower soil bulk density due to continuous manure application and buffering capacity of sand with higher porosity. So, continuous management of manure for apple production can be of great value (Zuoping et al., 2014). Previous research in other crops has shown that providing organic fertilizers can improve plant growth and development (Kai et al., 2020a; Kai et al., 2020b). Therefore, reduced rates of nitrogen release by organic fertilizers can cause nitrogen deficiencies that could cause an increased accumulation of phenolic flavonoids in some fruits affecting their flavor and nutritional quality (Lester & Saftner, 2011). In apple, the administration of fertilizers significantly

influenced the level of several chemical compounds related to taste and aroma, such as the level of sugars and organic acids or some volatile substances responsible for aroma. Significant differences were observed in several sensory attributes in apples as a result of different fertilization systems (Raffo et al., 2014). Thus, due to the higher doses of K, a higher level of organic acids was obtained, which has the consequence of changing the taste due to the change in the ratio of sugar/organic acids (Dodocioiu & Buzatu, 2023). Research results show that biological fertilization can be a sustainable and economically efficient alternative to standard fertilization (NPK) in apple production. Testing a large number of products have shown a positive influence on vegetative growth and productivity in apple. The impact of biopreparations on the quantity and quality of the harvest is observed after more time of application (Mosa et al., 2015). A sensory evaluation of horticultural products obtained from organic crops showed an improvement in sugar content and aromatic substances (Liu, 2012).
Therefore, the aim of the present study was to investigate the effects of organic manure and other organic fertilizers applied radicularly or extraradially on the quality of apple in the apple orchard in Argeșului meadow, southern Romania.

MATERIALS AND METHODS

Experiment location and plant material
The study was carried out in 2023 in a plantation located in southern Romania (44°54'n, 24°52'e) of a private farm, using a seven-year-old apple plantation as study material. Three apple varieties 'Romus 3', 'Idared' and 'Golden Delicious' were tested.
Soil description
The apple crop is on a brown-clay soil with a clay-clay texture in the first 70 cm.
Plant fertilization
Fertilizers were applied in a completely randomized block design with 3 plants per plot with a 3-plant spacing between treatments with three replicates. The experiment was bifactorial. Factor A, apple (*Malus domestica*) had 3 levels: 'Romus 3', 'Idared' and 'Golden Delicious'. Factor B: five different fertilization treatments according to the scheme shown in Table 1.

Table 1. Fertilization variants and applied fertilizer doses

Fertilization variant	Treatment applied		Application time	
	Soil	*Foliar*	*Soil*	*Foliar*
V1	-	-		
V2	Biohumus (0.5 L/ha)	Macys BC (2 L/ha) Cifamin BK (1 L/ha)	Spring (when flowering) Autumn (after the leaves fall)	-after flowering -young fruit
V3	Biohumus (0.7 L/ha)	Macys BC (2 L/ha) Cifamin BK (1 L/ha)		
V4	Biohumus (0.9 L/ha)	Macys BC (2 L/ha) Cifamin BK (1 L/ha)		
V5	Manure (20 t/ha)	-	Autumn (after the leaves fall)	

Biohumus is a 100% organic fertilizer, an active humic preparation, purely ecological. It contains amino acids, salts of humic acids, fulvic acids, humic acids, micro and macro nutrients, live bacteria and other compounds easily absorbed and assimilated by plants.
Cifamin BK is a preparation, very rich in organic compounds with biostimulating action on the physiology of plants, recommended especially for improving the size of fruits and vegetables. It contains amino acids of vegetable origin derived from enzymatic hydrolysis (tryptophan, arginine).
Macys BC is an organic product recommended for foliar administration based on *Macrocystis integrifoglia*. It provides plant-essential algae extracts with direct action on plant physiology, such as natural growth regulators and carbohydrates such as alginic acid.
Fruit quality
The determinations were carried out in 2023, on fruits harvested by hand at the technical harvest maturity. The following quality indicators were determined: fruit mass, firmness, color, soluble dry matter content, total titratable acidity, total sugar content and total polyphenol

content. All determinations were performed in three replicates for each cultivar with the fertilization variants.

Fruit mass, expressed in g/fruit, was determined by weighing 20 fruits from a sample and calculating the average weight of a fruit,

Fruit firmness (expressed in HPE units) was determined using a non-destructive Qualitest HPE penetrometer.

***Total dry matter content* (DM)**

Total dry matter content was determined by a gravimetric method that consists of drying 10 g of fruit tissue at 105°C to constant weight, according to Gergen (2004).

Total soluble solids (TSS) were determined using a refractometer.

Total acidity (TA)

The total acidity expressed as malic acid (%) in apples was determined by the titrimetric method in an aqueous extract neutralized with a 0.1 N NaOH solution using phenolphthalein as an indicator.

Dosage of total sugar

The total sugar content, expressed as a percentage (%), was estimated by the method of Fehling-Soxhlet, 1968 (JAOAC, 1968). The principle of this method consists in the oxidation reaction between the copper in the copper alcoholate of sodium potassium tartrate and the aldehyde and ketone groups of the reducing sugars.

The method allows the determination of the amount of sugar that reduces a certain volume of Fehling's reagent.

Vitamin C content

The dosage of ascorbic acid in the fruits (expressed in mg/ 100 g FW) was carried out, in an acidic environment, by the oxidation of L-ascorbic acid to dehydroascorbic acid in the presence of a blue dye (2,6-dichloroindophenol) followed by the reduction of the dye to the form colorless, and at pH 4.2 it turns red at (PN-A-04019: 1998).

Total polyphenol content (TPH)

Fruit TPH was assayed using Folin-Ciocalteu reagent (Singleton et al., 1999) and was measured at 760 nm wavelength. Gallic acid was used as a standard and the results were expressed in mg GAE/kg FW.

Statistical analyses

An IBM SPSS 16 program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The obtained results were subjected to a one-way analysis of variance (Anova). The Duncan Multiple Range test was used to determine the degree of significance. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The physical indicators of the fruits (mass, firmness) were determined on fresh fruits, immediately after harvesting.

The average weight of apple fruits varied between 111.17 g (variety 'Romus 3', V1) and 147.41 g (variety 'Idared', V4) (Table 2). The variety "Idared" stood out with the largest fruits in the case of all fertilization options.

Table 2. Physical indicators of apple fruit quality (V1-V5)

		V1	V2	V3	V4	V5	Average of cultivars
Weight (g)	Romus 3	111.17±13.17c	119.02±24.24c	123.94±3.82c	169.12±4,70b	145.69±11.70c	133.03±22.58c
	Idared	161.81±18.18a	195.25±37.57a	212.78±29.02a	247.41±34,71a	236.90±34.33a	207.45±43,40a
	Golden Delicious	131.48±19.98b	163.58±15.55b	170.32±18.23b	184.23±20,45b	188.51±20.77b	165.38±27.77b
Firmness (unit HPE)	Romus 3	76.14±3.11a	70.97±5.35b	74.12±7.03b	71.93±4.04c	74.83±4.06b	74.08±4.73c
	Idared	77.16±1.71a	76.35±2.10a	75.54±1.72b	76.12±2.28b	76.10±2.36b	76.29±2.03b
	Golden Delicious	78.94±3.61a	79.47±3.38a	80.19±2.49a	81.74±1.67a	81.73±1.41a	80.28±2.91a

Figure 1 shows that the best results were obtained with option 4 – Biohumus 0.9 l/ha root, Macys BC (2 L/ha) and Cifamin BK (1 L/ha) extraroot. The results show that the supply of organic fertilizers has a positive effect on the average fruit weight. Tamara et al. (2005), Grzyb et al. (2012) observed an increase in average fruit weight in organic farming as a result of the application of organic fertilizers. It may also be influenced by the type or frequency of treatments provided.

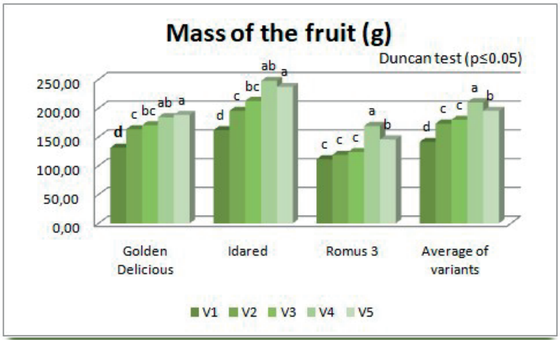

Figure 1. The effect of the applied treatments on the average weight of the fruits (g)

Apple pulp firmness is a particularly important parameter regarding the quality and durability of production in the marketing system. According to the results obtained (Table 2), fruit firmness was significantly influenced by the genetic characteristics of the cultivar. In the variety 'Golden Delicious' the fruits were firmer compared to the varieties 'Romus 3' and 'Idared'. According to the results obtained, this parameter did not vary significantly following the provision of organic fertilizers. In the 'Golden Delicious' variety, an improvement in the firmness of the fruits was observed in the dynamics with the increase in the doses of fertilizer applied to the roots. This tendency was not confirmed in the fruits of the summer variety 'Romus 3' or the late ripening variety 'Idared' (Figure 2).

Determining the biochemical composition of fruits is very important for determining the quality of horticultural products. This influences not only the taste of the fruit, but also its health-promoting properties.

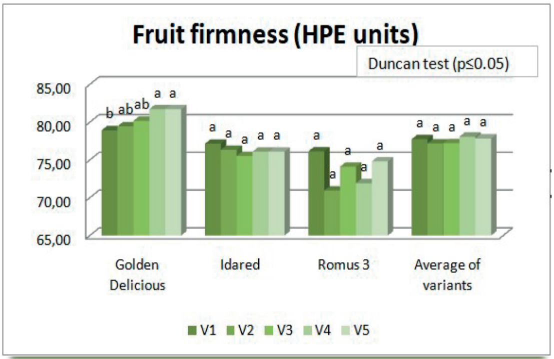

Figure 2. The effect of the applied treatments on the fruit firmness (HPE units)

According to the obtained results, the fruits of the 'Idared' variety had a higher level of TSS in their composition (Figure 3). Moreover, it was shown that all applied organic treatments affected the TSS of the fruits of this variety. On the cultivar average, TSS (Figure 3) was higher in the case of the control variant (V1) and variant 3. Arabloo et al. (2017), Ilie et al. (2017) showed that foliar application of organic fertilizers increased fruit TSS and yield quality.

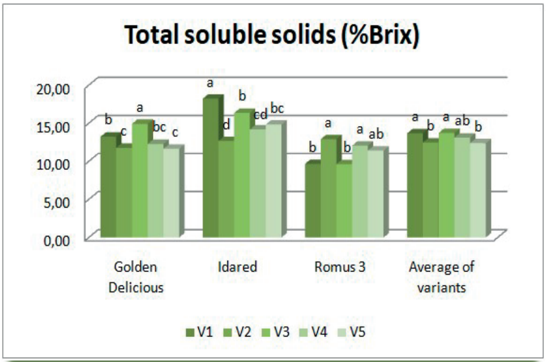

Figure 3. The effect of the applied treatments on the total soluble solids (%Brix)

The study showed that DW also differed depending on the genetic characteristics of the cultivar. Analogous to TSS, DW was higher in the cultivar 'Idared'. The percentage of DW was generally higher in V3 (Biohumus - 0.7 L/ha) and V4 (Biohumus - 0.9 L/ha) variants. The behavior of the summer variety 'Romus 3' was different from the behavior of the autumn varieties following the administered fertilizers. In this variety, an increase in DW dynamics was observed with increasing doses of Biohumus. In the case of manure administration, the increase of this biochemical quality indicator was insignificant. The autumn variety 'Idared', on the other hand, had a higher DW in the case of the variant fertilized with manure 20 t/ha (Figure 4). In conclusion, to increase this quality indicator, the root administration of Biohumus fertilizer in doses greater than 0.5 L/ha is recommended.

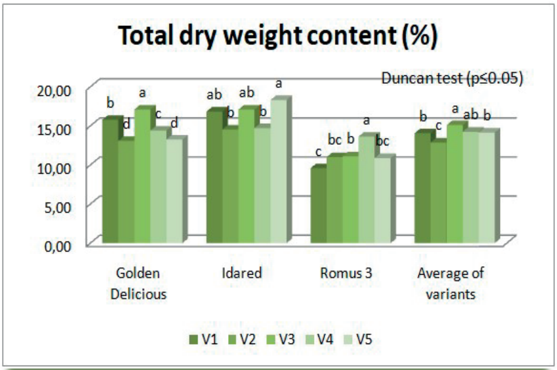

Figure 4. The effect of the applied treatments on the total dry weight content (%)

Titratable acidity (TA%) of fruits and vegetables depends on several factors, including genotypic differences, climatic conditions before harvest, as well as cultivation technologies (Lee & Kader, 2000). It is known that in colder areas or during periods of heavy precipitation, TA values become higher (Gherghi et al., 1986).

The dynamics of TSS accumulation in apple fruits was identical to that of DW. Testing the influence of the experimental factors (fertilization variant and variety) on fruit acidity in the three varieties studied, we emphasize the following (Figure 5): - The varieties 'Idared' and 'Romus 3' recorded a higher content of malic acid in the fruits. The biosynthesis of organic acids was significantly influenced by genetic characteristics. In the 'Idared 'variety, there was a slight decrease in AT with the increase in the doses of applied organic fertilizers. However, on the average of the variants, the highest values of the content of organic acids were found in the V3 variant (Biohumus 0.7 L/ ha) followed by variant V5 (manure 20 t/ha). A possible increase of this quality indicator in some apple varieties was also reported by Ilie et al. (2017) in organic crops.

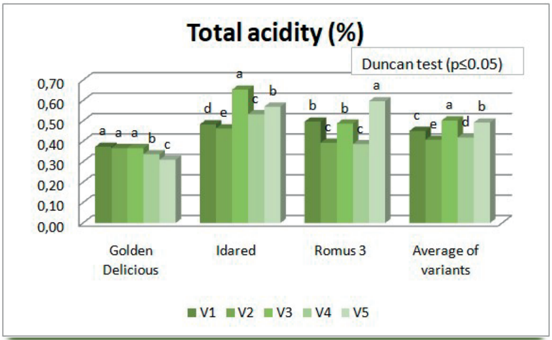

Figure 5. The effect of the applied treatments on the content of organic acids in fruits

In apples, sugars determine the organoleptic quality, especially the taste. Gündoğdu (2019) reported that climatic conditions and culture and rootstock technologies can influence the level of biochemical compounds such as sugar, phenolic compounds and organic acids in fruits. The total sugar content varied between 6.01% ('Romus 3' - V1) and 12.42% ('Golden Delicious' - V5). The 'Golden Delicious' variety had the highest sugar content, significantly higher than the other two varieties tested. The analysis of variance (Anova) showed a significant effect determined by the cultivar of 94.1% and of the treatments applied by 97.2% on the sugar content of the fruits of the studied apple cultivars.

In the summer variety 'Romus 3' (Figure 6), the maximum sugar content was obtained in the case of variant 4 (Biohumus 0.9 l/ha) and in the autumn varieties in the variant consisting of 20 t/ha of manure (V5). In addition to the quality of taste, the level of sugary substances in merestabiles also determines their caloric value.

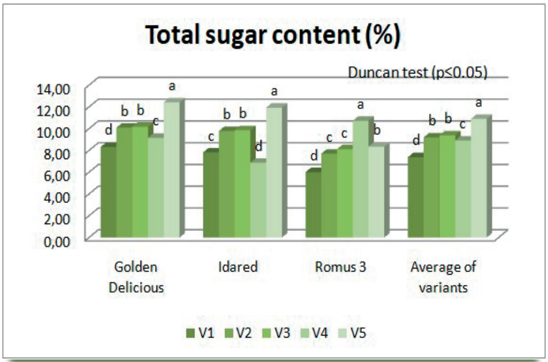

Figure 6. The effect of the applied treatments on the total sugar content of the fruits (%)

A negative interdependence between vitamin C content and nitrogen fertilizer rates supplied has often been reported (Weston & Barth, 1997 and Lee & Kader, 2000). And in the present experiment, the concentration of vitamin C in apples decreases with the increase in the dose of applied fertilizers (Figure 7). The minimum concentration of vitamin C was obtained after fertilization with manure (V5).

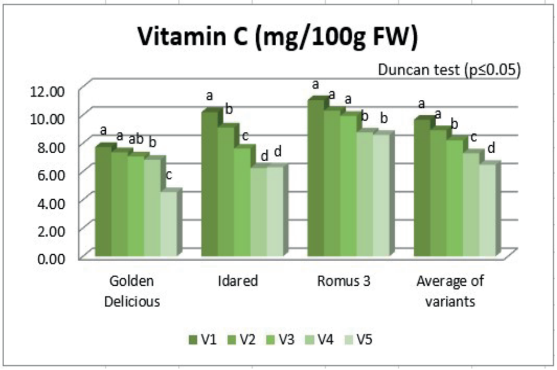

Figure 7. The effect of the applied treatments on the vitamin C content of the fruits (mg/100 g FW)

Phenolic compounds can contribute to the aroma of horticultural products and can be responsible for the sweet, bitter, pungent or astringent taste of fruits. Simple (volatile) phenols have a special role in establishing aroma (Tomás-Barberán & Espín, 2001). The results for TPH in the fruit of the apple cultivars analyzed varied between 1076.81 mg GAE/kg FW ('Idared' - V4) and 2230.44 mg GAE/kg FW ('Romus 3' - V3). Valavanidis et al. (2009) showed concentrations ranging from 800-1960 (mg GAE⁄ kg FW). Higher values were reported by Imeh and Khochar (2002), in the range of 3000-5350 (mg GAE⁄kg FW). The influence of applied fertilizer rates on fruit TPH was inconsistent. The data from the specialized literature, most of the time, were inconclusive Young, et al. (2005). According to Rosa et al. (2007), an explanation of the influence of culture technology on the biosynthesis of secondary metabolites in fruits is difficult due to the multitude of factors that affect their concentration.

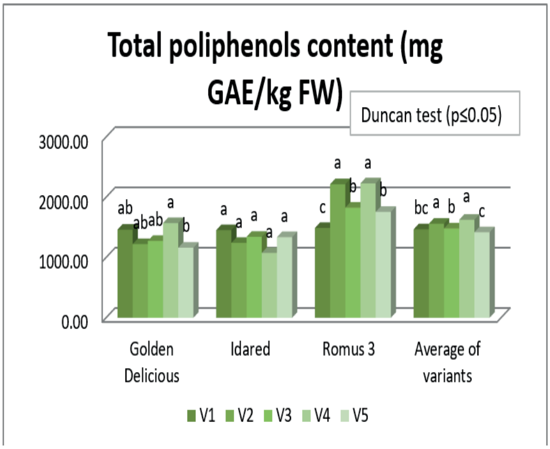

Figure 8. The effect of the applied treatments on the total poliphenols content of the fruits (mg GAE/kg FW)

Following the results obtained in the tests performed on the fruits of the three apple varieties analyzed regarding the quality parameters, some positive or negative, statistically significant interdependencies were obtained between most of the studied indicators (Table 3).

Table 3. Correlation between the values of the quality indicators studied in the fruits of the 'Romus 3', 'Idared' and 'Golden Delicious' apple cultivars fertilized with organic products.

	Mass of the fruit (g)	Firmness (HPE units)	TSS (%Brix)	DW (%)	TA (%)	Total sugar (%)	Vitamin C (mg/100g FW)	TPH (mg GAE/kg FW)
Mass of the fruit (g)	1							
Firmness (HPE units)	-0,051	1						
TSS (%Brix)	0,376(**)	0,138	1					
DW (%)	0,537(**)	0,263	0,780(**)	1				
TA (%)	0,316(*)	-0,308(*)	0,275	0,121	1			
Total sugar (%)	0,333(*)	0,153	0,322(*)	0,432(**)	-0,243	1		
Vitamin C (mg/100g FW)	-0,086	-0,526(**)	-0,218	-0,388(**)	0,221	-0,267	1	
TPH (mg GAE/kg FW)	-0,522(**)	-0,426(**)	-0,160	-0,413(**)	-0,119	-0,202	0,358(*)	1

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

The obtained data demonstrated a positive linear correlation, distinctly significant, between the fruit weight of the analyzed cultivars and TSS (r = 0.376), between fruit weight and DW content (r = 0.537) and a negative linear interdependence between fruit weight and TPH content (r = -0.522).

A negative linear relationship was also obtained between fruit firmness and ascorbic acid level (r=-0.526), between fruit firmness and TPH concentration (r = -0.426; p<0.01).

The relationship between TSS and DW was positive, distinctly significant, with a high intensity (r = 0.780).

The correlation between DW level and total sugar content was positive, distinctly significant (r = 0.432) and the relationship between DW and vitamin C or TPH content was linearly negative (r =-0.388 or r = -0.413).

CONCLUSIONS

Physical quality parameters (fruit weight and firmness) indicated variations determined by genetic diversity. The applied treatments did not significantly influence the firmness of the fruits.

The fruit weight of the three apple cultivars analyzed improved with the increase in the doses of fertilizers supplied.

It was observed that the level of organic compounds in the fruits of the studied apple varieties depends mainly on the genetic characteristics of the cultivar and the nature and doses of fertilizers administered.

The provision of organic products in apple crops influences the taste of the fruit (it improves the level of carbohydrate compounds and influences the biosynthesis of organic acids). A negative influence on the biosynthesis of ascorbic acid determined by increasing the doses of administered fertilizers was noted.

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RESEARCH ON THE INFLUENCE OF ORGANIC FERTILIZERS ON THE AGROCHEMICAL INDICATORS OF THE SOIL

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Abstract

The aim of this study was to evaluate the influence of organic fertilizers on some soil agrochemical indicators, such as: soil reaction (pH), content of soil in organic carbon, humus, total nitrogen, total phosphorus and total potassium. Research was carried out in an apple demonstrative plot established in 2010 at Maracineni, in a private farm from Arges County. In 2022 the following fertilization variants were applied: V1 - Unfertilized; V2 - Biohumus – 0.5 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; V3 - Biohumus – 0.7 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; V4 – Biohumus – 0.9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; V5 – manure – 20 t/ha. The following determinations were carried out: pH in aqueous solution mix soil:water = 1:2.5 (potentiometric method); organic carbon according to the Walkley Black method, respectively the humus by calculation; the total nitrogen was determined by the Kjeldahl method; the total phosphorus was determined spectrophotocolorimetric, the Egner-Riehm-Domingo method; the total potassium was determined flamphotometrically from the same extract obtained when determining the phosphorus. Fertilization with manure in a dose of 20 t/ha (V4), has led to an improvement of soil acidity, increased soil content in humus, nitrogen, phosphorus and potassium compared to the unfertilized variant and with the other fertilization variants.

***Key words**: humus, nitrogen, phosphorus, potassium, soil reaction.*

INTRODUCTION

Apple is an important fruit crop in many countries, including Romania. The area cultivated with apples in 2022 was 54,070 ha, which ensured a production of 543,380 tons (Faostat, 2024). This fruit production ranks apple on the 2nd place in Romania, after plum. However, Romanian apple production is lower than other countries like the USA, New Zealand and China etc. due to the cultural practices. Some authors report that low productivity is closely related to low soil fertility (Krishna, 2014; Kai & Kubo, 2020; Kai & Adhikari, 2021). The culture of the apple in the ecological system can reduce the amount of chemical fertilizers that damage the structure of the soil. Organic fertilizers application improved both plant growth and soil conditions (less acid soil and more soil carbon) (Kai & Adhikari, 2021). Kai & Kubo, in 2020, conducted a study in organic apple orchards in Japan and reported high levels of carbon, nitrogen, phosphorus and potassium in the soil, which can improve bacterial biomass and leading to enhanced N and P circulation. In conclusion, organic fertilizer management system can improve soil nutrient contents.

In Romania, ecological apple production is still quite limited (Butac et al., 2021), due to the lack of organic fertilizers, which limits profitability of ecological apple orchards. McArtney & Walker, in 2004 also reports a lack of organic fertilizers in Oceania. In previous articles it is specified that production can be lower by about 30% in the situation of using organic methods (Reganold et al., 2001; de Ponti et al., 2012).

The aim of this study was to evaluate the influence of organic fertilizers on some soil agrochemical indicators, such as (soil reaction - pH, content of soil in organic carbon, humus, total nitrogen, total phosphorus and total potassium in an apple orchards managed under ecological system in Maracineni – Arges area.

MATERIALS AND METHODS

Field trial

Research was carried out in an apple demonstrative plot established in 2010 at Maracineni, in a private farm from Arges County.

Fertilization variants

In 2021-2022 the following fertilization variants were applied: V1 – Unfertilized; V2 – Biohumus – 0.5 L/tree, soil application + Macys BC 28 – 2 L/ha, foliar application + Cifamin BK – 1 L/ha, foliar application; V3 – Biohumus – 0.7 L/tree, soil application + Macys BC 28 – 2 L/ha, foliar application + Cifamin BK – 1 L/ha, foliar application; V4 – Biohumus – 0.9 L/tree, soil application + Macys BC 28 – 2 L/ha, foliar application + Cifamin BK – 1 L/ha, foliar application; V5 – Manure – 20 t/ha.

A short description of fertilizers and application period

Biohumus is a 100% organic fertilizer, produced with the help of earthworms, which stimulates the yield, growth and health of trees (Butac & Chivu, 2020). In the apple experimental filed, soil fertilization with Biohumus was carried out in increasing doses, from 0.5 L/tree, 0.7 L/tree to 0.9 L/tree in two moments: in spring before the start of vegetation and in autumn after the fall of the leaves (in 2022).

Macys BC 28 is a fertilizer based 100% on the algae *Macrocystis integrifolia*, which stimulates root development, vegetative growth, flowering and fruiting, and also the fruits size and quality (Butac & Chivu, 2020). Macys BC 28 fertilizer was applied foliar in doses of 2 L/ha, in two moments: after flowering and in the young fruit phase (in 2022).

Cifamin BK is a special fertilizer based also on the algae *Macrocystis integrifolia*, very rich in organic components, indicated for improving the size and fruits quality, keeping fruit and firmness unaltered, ensuring optimal shelf-life (Butac & Chivu, 2020). Cifamin BK fertilizer was also applied foliar in doses of 1 L/ha, also in two moments: after flowering and in the young fruit phase (in 2022).

Foliar fertilizers, Macys BC 28 and Cifamin BK were dissolved in 500 L water.

Manure from cattle is a natural fertilizer aerobically fermented for 12 months. It was applied in November 2021.

Agrochemical indicators of the soil

In autumn 2021, before the applied the fertilization, soil samples were collected from the depth of 0-20 cm and 20-40 cm and chemical analyzes were carried out (acidity, content in humus, organic carbon, nitrogen, phosphorus and potassium). It was concluded that the respective soil, on which a conventional technology was applied, has a weak acid reaction, low humus content in the arable horizon, low nitrogen and phosphorus and moderate potassium content (Table 1).

Table 1. Agrochemical indicators of the soil

Depth (cm)		0-20	20-40
Acidity Indicators	pH	6.37	6.14
Fertility indicators	Total nitrogen (%)	0.08	0.07
	Phosphorus P_2-P_2O_5 (ppm)	7-15	6-10
	Potassium K-K_2O (ppm)	130	85
	Organic carbon (%)	0.95	0.83
	Humus (%)	1.64	1.43

Soil sampling and analysis of chemical properties

Soils were sampled in November 2022 using a boring stick. Soil samples of the top 20 cm were taken from around the base of three trees for analyzing the chemical properties. The soil chemical properties were analyzed using the methods described by Florea et al. (1987). The following analyses were carried out on the fresh soil samples: soil pH was measured in a soil:water suspension (ratio 1:2.5 w/v) by the potentiometric method; organic carbon with wet oxidation method followed by titrimetric dosing by Walkley – Black method modified by Gogoașă, respectively the humus calculated with the formula: humus %= C org x 1.724 (Rusu & Mărghitaș, 2010); the total nitrogen was determined by Kjeldahl method (Kjeldahl, 1883); the total phosphorus was determined spectrophotocolorimetric by Egner-Riehm-Domingo method by which the phosphates are extracted from the soil sample with a solution of acetate - ammonium lactate at pH=5.75, and determined colorimetric phosphate anion extracted as molybdenum blue (Egnér et al., 1960); the total potassium was determined flamphotometrically by which the hydrogen and ammonium ions of the extraction solution replace by exchange the exchangeable potassium ions in the soil sample which are thus passed into the solution (Egnér et al., 1960).

Statistical analysis

Results were processed by Excel (Microsoft Office 2010) and SPSS Trial Version 14.0. Data were subjected to analysis of variance (One-way ANOVA; p ≤ 0.05), and Duncan's Multiple Range Test (DMRT) post hoc tests were used to measure specific differences between sample means.

RESULTS AND DISCUSSIONS

***Reaction of the soil* (pH)**

In general, most cultivated plants prefer soils with neutral, slightly acidic or slightly alkaline pH (6.3-7.5). The consumption of nutrients by fruit trees depends on the pH. For example, calcium and magnesium are easily assimilated by fruit trees at pH 7-8.5, nitrogen at pH 6.0-6.8, phosphorus at 6.5-7.5 and potassium at higher pH of 6 (Nagy et al., 2022).

The values of pH are between 5.83 (V4) and 6.96 (V5), with a mean value of 6.17 (Figure 1).

Fertilization with Biohumus in doses 0.5 L/tree (V2), 0.7 L/tree (V3) and 0.9 L/tree (V4) (soil application), associated with Macys BC 28-2 L/ha and Cifamin BK - 1 L/ha (both fertilizers with foliar application) has led to a significant decrease in pH values compared with unfertilized variant.

Fertilization with manure in a dose of 20 t/ha (V5), has led to an improvement of soil acidity. Significant increases, statistically insured, are observed, from 6.27 in the V1 – unfertilized variant, to 6.96 in the fertilized variant with manure (V5) (Figure 1).

In previous studies performed in apple orchards managed in the organic system, more or less close values of the pH have been obtained. The effect of cattle manure on the improvement of the soil reaction was reported in Romania by Borlan & Hera many years ago (1973). Recent, in Romania, in an apple orchard in which compost from urban sludge was applied, the pH values between 6.5 and 7 were obtained (Nagy et al., 2022). In Japan, Kai & Ahikari, reported values of soil pH of 6.9 in organic apple system. In Italy, Bona et al. (2022) reported high values of soil pH in apple orchard fertilized by matured manure (about 8.8).

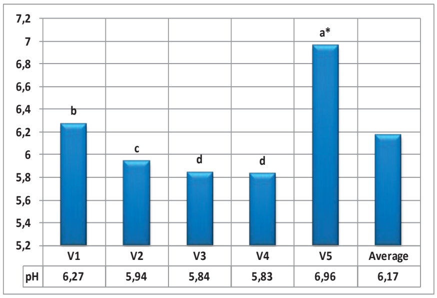

Figure 1. The influence of organic fertilization on the pH of the soil

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

***Content of soil in organic carbon* (%)**

As expected, the content of soil in organic carbon increased significantly with the increase of doses of fertilizer.

There are significant differences between all fertilized variants. In the fertilization variant with manure (V5), the value of the carbon organic has registered the highest increases compared to all other variants.

It is noted that the dose of manure is much higher than the doses of Biohumus, Macys BC 28 and Cifamin BK applied in the other fertilization variants (Figure 2).

Similar results were reported by Kai & Adhikari in 2021.

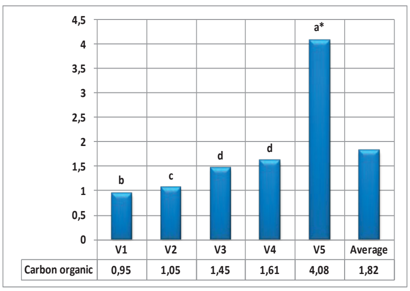

Figure 2. The influence of organic fertilization on the content of soil in the organic carbon

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Content of soil in humus **(%)**

Humus has a sedimentation effect on the soil (Belopukhov et al., 2023).

In our experiment we observed a tendency of increases of humus content in the soil. Similar results are reported by the other authors (Kopytko et al., 2017; Elkhlifi et al., 2022; Belopukhov et al., 2023; Butkevičienė et al., 2023).

The values of humus were between 1.64 (V1) and 7.04 (V5), with a mean value of 3.15 (Figure 3).

It can be seen from Figure 3 that, in the case of fertilization with manure (V5), content of soil in humus was much higher than in the other fertilization variants (7.04% versus 1.64% in V1 – unfertilized, 1.81% in V2, 2.50% in V3, respectively 2.78% in V4 (Figure 3).

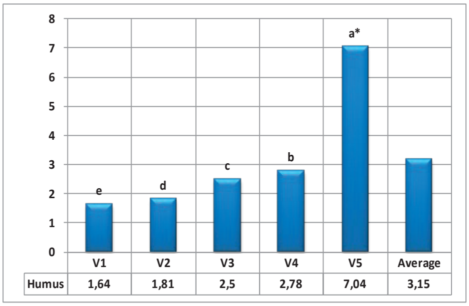

Figure 3. The influence of organic fertilization on the content of soil in the humus

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Content of soil in total nitrogen **(%)**

Also, the content of soil in the total nitrogen was higher in the case of fertilization with manure (V5). The differences are significantly much higher than all the other variants, including the unfertilized variant (Figure 4).

The positive effect of the manure on the content of the soil in nitrogen has been reported by the Khusaynov et al. in 2019 in Russia, the dose of manure being 30 t/ha.

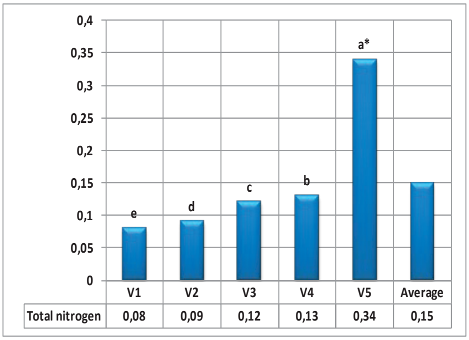

Figure 4. The influence of organic fertilization on the content of soil in the total nitrogen

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Content of soil in total phosphorus P_2-P_2O_5 **(ppm)**

The values of phosphorus were between 7 ppm (V1) and 162.33 ppm (V5), with a mean value of 60.26 ppm (Figure 5).

The total phosphorus content has increased with the increase of the doses of Biohumus and also in the variant fertilized with manure.

The differences are significantly higher than the unfertilized variant and the differences are also significant between the fertilization variants with different doses of organic fertilizers (Figure 5).

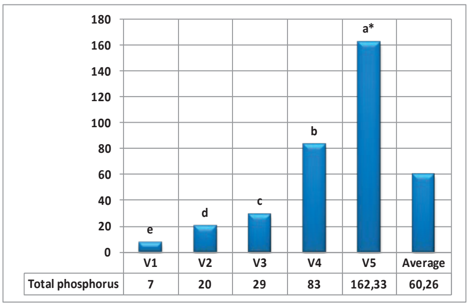

Figure 5. The influence of organic fertilization on the content of soil in total phosphorus

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Similar results regarding the influence of organic fertilization on content of soil in phosphorus are reported by the other authors (Khusaynov et al., 2019; Belopukhov et al., 2023).

***Content of soil in total potassium K-K₂O* (ppm)**

Information about the content of potassium is important in the agrochemical analysis of soils (Belupukhova et al., 2023).

The values of potassium were between 100 ppm (V1) and 552.33 ppm (V5), with a mean value of 205.26 ppm (Figure 6).

The potassium content increased in soil with increases of organic fertilizers doses applied to the soil. Practically the variant 5 – fertilization with manure cannot be compared to the other organic fertilization variants.

However, there are significantly differences between V3 and V4 variants compared to the unfertilized variant and significantly higher values in V4 compared to V3. The increase of the potassium content in V2 is insignificant to the unfertilized variant (Figure 6).

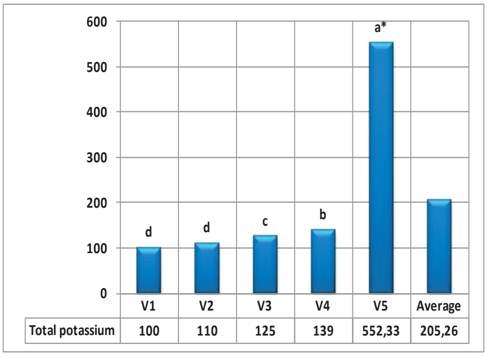

Figure 6. The influence of organic fertilization on the content of soil in total potassium

*Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Based on the data obtained in this study regarding content of soil in potassium, we confirmed that the our results are similar with other results obtained by Khusaynov et al. (2019), Kai & Adhikari (2021), Butkevičienė et al. (2023).

CONCLUSIONS

Fertilization with Biohumus in doses 0.5 L/tree (V2), 0.7 L/tree (V3) and 0.9 L/tree (V4) (soil application), associated with Macys BC 28 – 2 L/ha and Cifamin BK – 1 L/ha (both fertilizers with foliar application) has led to a significant decrease in pH values compared with unfertilized variant.

Fertilization with manure in a dose of 20 t/ha (V5), has led to an improvement of soil acidity versus the unfertilized variant and to all other organic fertilization variants.

The organic carbon content has increased significantly with the increase of the fertilizers doses, the highest increase being obtained in the case of fertilization with 20 t/ha of manure.

The contents of soil in N, P, K have increased with the increase of the doses of fertilizer, the highest increases being obtained in the case of fertilization with manure.

The results of our study show that organic fertilization in apple culture had a good effect on the content of soil in humus, carbon, nitrogen, phosphorus and potassium.

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FRUIT QUALITY ASSESSMENT IN RASPBERRY BREEDING

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Abstract

The available raspberry cultivars are rapidly changing, highlighting the need for improvement in raspberry cultivars. Fruit quality assessment is crucial for red raspberry assortment breeding, with being essential for consumer acceptance. This study aimed to characterize raspberry fruits from different progenies morphologically. The data collected included average fruit weight, shape index, colour and soluble solids. The results showed significant variation in fruit weight and soluble solids content among the progenies. The fruit weight ranged from 2.50 g/fruit ('16-22-4') and 3.52 g/fruit ('16-22-5') and the soluble solids content: 11.20°Brix ('16-23-20') and 17.30°Brix ('16-1-23'). Based on the fruit quality, certain genotypes were identified as promising for future steps in the breeding program, including '16-1-10', '16-1-11', '16-1-22', '16-22-5', '16-22-11' and '16-23-20'.

Key words*: Rubus idaeus, selection, fruit weight; soluble solids content; colour.*

INTRODUCTION

Rubus is one of the most diverse genera in the plant kingdom, with over 400 species (Bailey 1949) classified into 12 subgenera (Jennings, 1988). The cultivated subcategories include raspberries, blackberries, arctic fruits, and flowering raspberries, all of which have been used in breeding initiatives. The key varieties are the European red raspberry (*R. idaeus* L. subsp. *idaeus*), the North American red raspberry (*R. idaeus* subsp. *strigosus* Michx), and the black raspberry (*R. occidentalis* L.). Raspberry is believed to have originated from the Ide mountains in Turkey. *Rubus* species are low-growing to upright shrubs with thorns, producing new shoots from the ground (called canes). They are perennials because each bush comprises biennial canes that overlap in age. The leaves are compound with 3-5 leaflets, with the middle one being the largest, and the edges are serrated to irregularly toothed (Graham et al., 2007). The raspberry belonging to the *Rosaceae* family and widely cultivated in Asia (Veljkovi´c et al., 2019), are recognized for their exceptional cold and disease resistance, nutritional value, and flavor, making them widely available in the market (Xian et al., 2019). Ongoing research is focused on exploring their components and effectiveness. These small, soft fruits are rich in nutrients, including sugars, organic acids, vitamins, and phytochemical compounds (Vara et al., 2019). Approximately 50 active raspberry breeding programs are currently operating in 26 countries, with the majority located in Europe and North America (Kempler et al., 2012). Raspberry breeding programs for the fresh market aim to achieve fruit quality, productivity, and resistance to pathogens. The plant material forms the foundation for breeders' work, and new cultivars must be well-suited to the environmental conditions where they will be cultivated. Additionally, agronomy and the influence of different cultivation systems on plant behavior should be taken into account.

Therefore, breeders must consider genetics, environment, and agronomic technologies to develop new cultivars (F.R. Luz et al., 2022). Evaluating the quality of red raspberries is crucial, especially for product development and breeding programs, focusing on their physical and chemical characteristics. Genetic breeding of fruit yield is a primary goal in raspberry breeding programs globally (Way et al., 1983). While breeders have already made significant progress in increasing yield, there is still potential for further improvement in crops like raspberry, which have relatively short breeding histories (Jennings, 1988).

The consumer market has seen a growing interest in healthy eating and natural products in recent years, with raspberry aligning perfectly with these trends, leading to increased significance (Sawicka et al., 2023).

MATERIALS AND METHODS

Plant Material

The researches were carried out in 2021–2023 at the Research Institute for Fruit Growing (RIFG), Pitesti Romania (44°54’12” Northern latitude, and 24°52’18” Eastern longitude, 284 m altitude) in open an experimental field. There were twenty-three selections obtained in the year 2016 and one cultivar ('Heritage') was used as control in the evaluation (Table 1) in the randomized block design with three repetitions plots (10 plants/ genotype/ repetition).

Experiment Scheme

The selection evaluation plot was planted in April 2020 by using bare-root plants. Each genotype was planted twenty plants at distances 0.5 m from each other. The soil was soddy-podzolic clay loam showing medium and low humus content, the irrigation system used was the sprinkler irrigation type and plant protection treatments were applied. The field trials were in a conventional system, and before planting the following quantities were applied 40kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 sand 60 kg ha^{-1} K_2O, as basic fertilization.

Table 1. The origin of the genotypes

Genotype	Parentage
16-1-2	Heritage × Polka
16-1-5	Heritage × Polka
16-1-10	Heritage × Polka
16-1-11	Heritage × Polka
16-1-12	Heritage × Polka
16-1-13	Heritage × Polka
16-1-14	Heritage × Polka
16-1-16	Heritage × Polka
16-1-17	Heritage × Polka
16-1-21	Heritage × Polka
16-1-22	Heritage × Polka
16-1-23	Heritage × Polka
16-22-2	92-7-40 × Yan Yan
16-22-4	92-7-40 × Yan Yan
16-22-5	92-7-40 × Yan Yan
16-22-7	92-7-40 × Yan Yan
16-22-8	92-7-40 × Yan Yan
16-22-11	92-7-40 × Yan Yan
16-22-15	92-7-40 × Yan Yan
16-23-7	BL 4 × Polana
16-23-12	BL 4 × Polana
16-23-18	BL 4 × Polana
16-23-20	BL 4 × Polana
Heritage	[(Milton × Cuthbert) × Durham]

Fruit quality parameters

The measured indicators were recorded during the optimal fruit harvesting period from a sample of 50 fruits. All measurements and analysis were conducted with 3 replications. The raspberry fruits were harvested manually directly in plastic pans. The length and diameter of the fruit were determined by measuring the fruit using a digital caliper. The shape index of the fruit was calculated as the ratio of these two dimensions (Titirica et al., 2023). The total soluble solids content (TSS) was measured using a digital refractometer Haana Instruments 96801 and values were recorded in °Brix. The external fruit color was determined with a colorimeter Konica Minolta CR 400, based on system Huntel L*, a*, b* on both sides of the fruit (L* corresponds to brightness, a* and b* chromaticity coordinates from green to red and from blue to yellow, respectively). Chroma index was determined by the formula $C = (a^*2 + b^*2)^{1/2}$ and hue angle of the formula h^0 = arctangent (b*/a*), where 0^0= red-purple, 90^0= yellow, 180^0= bluish-green and 270^0 =blue (McGuire, 1992).

Statistical analysis was performed using the IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were statistically evaluated by analysis of variance (ANOVA), and Duncan’s multiple test range. Differences were considered statistically significant for values of $p < 0.005$.

RESULTS AND DISCUSSIONS

Table 2 shows the values for average berry weight, shape index and total soluble solids, with the indication of the values of mean and standard deviation for the 24 raspberry genotypes.

Berry weight is a key quality parameter in the commercial raspberry market. While consumers typically prefer large berries, excessive berry weight (potentially > 15.0 g) is generally not suitable for either processed or fresh market use (Clark and Finn, 2011). The average weight of the fruit is a genetically determined characteristic, influenced by technical and cultural conditions, and has shown different values over the three years of study. For the three years of study, berry weight oscillated between 3.52 for the '16-22-5' hybrid to 2.5 g for the '16-22-4' hybrid (Table 2).

The physical fruit properties of floricane fruiting raspberry vary in *height*, ranging from 16.33 mm for the '16-22-4' hybrid to 21.29 mm for '16-23-20'. The fruit *diameter* varied from 12.77 mm for the '16-1-21' hybrid to 20.50 mm for the '16-1-10' hybrid. *The shape index*, the mean oscillated from 1.31 for the '16-1-21' hybrid (as well as long conical fruit shape with larger values than 1.00) to 0.87 for the '16-22-7' hybrid. Similar results are reported by Milivojević, (2011), the values of fruit shape index ranged from 0.93 to 1.10.

The analysis of variance revealed significant variation in *the soluble solids content* among the hybrids and the control. Cv. 'Heritage' exhibited significantly higher soluble solids (17.40 °Brix) in comparison with the hybrids ($P < 0.01$).

The value of soluble solids oscillated between 9.30 °Brix for '16-1-10' hybrid and 17.29 °Brix for '16-1-23' hybrid.

The color of the fruit is a major component in determining its quality, as it is closely linked to the levels and types of anthocyanins present in the fruit (García-Viguera et al., 1998). Robbins and Moore (1990) and Haffner et al. (2002) found that the relative color differences between cultivars are preserved during storage. Fruit color and adhesion to the receptacle are the main indicators for producers to determine the optimal ripeness for harvesting. Similarly, consumers primarily rely on color to assess the quality of the fruit. Anthocyanins play a significant role in imparting the red color to raspberry fruits (Stavang et al., 2015).

Table 2. Fruit weight, size and total soluble solid characteristics of raspberry genotypes (average 2021-2023)

Genotype	Berry weight (g/fruit)	Height (mm)	Diameter (mm)	Shape index	Total soluble solid (^{0}Brix)
16-1-2	2.80±0.10^{fgh}	17.60±0.10^{e}	18.82±0.01^{f}	0.94±0.01^{hi}	15.20±0.10^{f}
16-1-5	2.51±0.01^{i}	16.83±0.01^{ghi}	18.74±0.01^{g}	0.90±0.01^{j}	11.60±0.10^{p}
16-1-10	3.44±0.01^{ab}	21.26±0.01^{a}	20.50±0.01^{a}	1.04±0.01^{d}	9.30±0.10^{r}
16-1-11	3.24±0.01^{c}	20.51±0.01^{b}	19.78±0.01^{c}	1.04±0.01^{d}	14.20±0.10^{i}
16-1-12	2.97±0.01^{de}	18.90±0.07^{c}	19.83±0.01^{c}	0.95±0.01^{fg}	12.40±0.01^{n}
16-1-13	2.74±0.01^{gh}	17.17±0.01^{efgh}	18.94±0.01^{d}	0.91±0.01^{ij}	13.10±0.10^{k}
16-1-14	2.92±0.01^{def}	18.34±0.01^{d}	18.34±0.01^{j}	1.00±0.01^{e}	16.60±0.10^{b}
16-1-16	2.84±0.01^{efgh}	17.58±0.01^{e}	19.45±0.01^{d}	0.90±0.01^{j}	14.50±0.010^{h}
16-1-17	2.83±0.01^{efgh}	17.57±0.01^{e}	18.66±0.01^{h}	0.94±0.01^{h}	16.40±0.10^{c}
16-1-21	2.76±0.01^{gh}	16.72±0.01^{hi}	12.77±0.01^{q}	1.31±0.01^{a}	16.01±0.10^{d}
16-1-22	3.01±0.01^{d}	20.01±0.01^{b}	18.47±0.01^{i}	1.08±0.01^{c}	14.70±0.01^{g}
16-1-23	2.85±0.01^{efgh}	17.18±0.01^{efgh}	18.50±0.01^{i}	0.93±0.01^{hi}	17.29±0.01^{a}
16-22-2	2.75±0.01^{gh}	17.21±0.01^{efgh}	17.77±0.01^{l}	0.97±0.01^{fg}	12.50±0.01^{mn}
16-22-4	2.50±0.01^{i}	16.36±0.01^{i}	17.25±0.01	0.95±0.01^{gh}	12.60±0.01^{k}
16-22-5	3.52±0.01^{a}	20.31±0.01^{b}	18.86±0.01^{f}	1.08±0.01^{c}	14.10±0.01^{m}
16-22-7	2.88±0.01^{defg}	17.31±0.01^{efg}	19.81±0.01^{c}	0.87±0.01^{k}	12.20±0.01^{o}
16-22-8	2.94±0.01^{def}	18.25±0.01^{d}	17.47±0.01^{m}	1.04±0.01^{d}	13.30±0.10^{j}
16-22-11	3.22±0.01^{c}	20.06±0.01^{b}	20.29±0.01^{b}	0.99±0.01^{ef}	11.50±0.01^{p}
16-22-15	2.80±0.10^{fgh}	19.01±1.15^{c}	18.49±0.01^{i}	1.03±0.06^{d}	15.40±0.01^{e}
16-23-7	2.71±0.01^{g}	17.16±0.01^{efgh}	17.40±0.01^{n}	0.99±0.01^{ef}	11.50±0.01^{p}
16-23-12	2.88±0.01^{defg}	17.34±0.01^{efg}	16.45±0.01^{p}	1.05±0.01^{cd}	14.20±0.01^{i}
16-23-18	2.70±0.01^{g}	16.93±0.01^{fgh}	17.75±0.01^{l}	0.95±0.01^{gh}	12.80±0.01^{l}
16-23-20	3.31±0.01^{bc}	21.29±0.01^{a}	18.17±0.01^{k}	1.17±0.01^{b}	11.20±0.01^{q}
Heritage	2.88±0.36^{defg}	17.45±0.78^{ef}	18.53±0.19^{i}	0.94±0.05^{gh}	17.40±0.19^{a}

Table 3. Fruit quality colour characteristics of raspberry genotypes (average 2021-2023)

Genotype	Brightness (L*)	Chromaticity a*- axis (red-green)	Chromaticity b*- axis (yellow-blue)	Chroma Index (C*)	The angle (h °)
16-1-2	25.69±0.01^{jk}	20.04±0.01^{ghi}	6.29±0.01^{hij}	20.70±0.61^{jk}	17.42±0.01^{ijk}
16-1-5	25.82±0.01^{hijk}	20.42±0.01^{gh}	6.60±0.01^{ghi}	21.46±0.01^{ghij}	17.9±0.01^{hij}
16-1-10	24.53±0.01^{l}	20.55±0.01^{fg}	6.72±0.01^{gh}	21.64±0.01^{fgh}	18.11±0.01^{hi}
16-1-11	27.90±0.01^{d}	23.37±0.01^{d}	8.28±0.01^{c}	24.79±0.01^{d}	19.50±0.01^{ef}
16-1-12	23.41±0.01^{m}	19.27±0.01^{jkl}	5.93±0.01	20.16±0.01^{kl}	17.10±0.01^{jk}
16-1-13	26.60±0.01^{fgh}	19.36±0.01^{jkl}	4.56±0.01^{l}	19.89±0.0^{l}	13.24±0.01^{m}
16-1-14	25.15±0.01^{kl}	15.07±0.01^{o}	4.36±0.01^{l}	15.69±0.01^{n}	16.12±0.01^{l}
16-1-16	26.73±0.01^{ef}	17.19±0.01^{n}	5.15±0.01^{k}	17.94±0.01^{m}	16.67±0.01^{kl}
16-1-17	26.52±0.01^{fghi}	19.89±0.01^{hij}	8.28±0.01^{c}	21.54±0.01^{ghi}	22.57±0.06^{c}
16-1-21	27.80±0.01^{d}	24.77±0.01^{ab}	8.05±0.01^{cd}	26.05±0.01^{b}	18.03±0.06^{hi}
16-1-22	25.71±0.01^{jk}	23.81±0.01^{cd}	7.72±0.01^{de}	25.03±0.01^{cd}	17.96±0.01^{hij}
16-1-23	25.18±0.01^{kl}	19.64±0.01^{ijk}	6.92±0.01^{fg}	20.83±0.01^{ijk}	19.42±0.01^{ef}
16-22-2	28.72±0.01^{c}	19.65±0.01^{ijk}	7.69±0.01^{de}	21.10±0.01^{hij}	21.38±0.01^{d}
16-22-4	25.38±0.01^{k}	16.64±0.01^{n}	6.98±0.01^{fg}	18.05±0.01^{m}	22.76±0.01^{c}
16-22-5	29.14±0.01^{bc}	20.63±0.01^{fg}	7.30±0.01^{ef}	21.89±0.01^{fg}	19.49±0.01^{ef}
16-22-7	27.43±0.01^{de}	18.51±0.01^{m}	6.16±0.01^{ij}	19.50±0.01^{l}	18.42±0.01^{gh}
16-22-8	31.72±0.01^{a}	19.77±0.01^{ijk}	9.30±0.01^{b}	21.85±0.01^{fgh}	25.20±0.01^{b}
16-22-11	25.87±0.01^{ghijk}	21.63±0.01^{e}	7.62±0.01^{de}	22.93±0.01^{e}	19.41±0.01^{ef}
16-22-15	29.81±0.01^{b}	23.74±0.01^{cd}	10.10±0.01^{a}	25.80±0.01^{b}	23.05±0.01^{c}
16-23-7	25.77±0.01^{ijk}	24.24±0.01^{bc}	8.46±0.01^{c}	25.67±0.01^{bc}	19.24±0.01^{efg}
16-23-12	26.32±0.01^{fghij}	18.92±0.01^{lm}	9.28±0.01^{b}	21.07±0.01^{hij}	26.13±0.01^{a}
16-23-18	26.66±0.01^{fg}	25.17±0.01^{a}	9.09±0.01^{b}	26.76±0.01^{a}	19.86±0.01^{e}
16-23-20	25.15±0.01^{kl}	21.13±0.01^{ef}	7.27±0.01^{ef}	22.35±0.01^{ef}	19.01±0.01^{efg}
Heritage	26.65±2.10^{fg}	19.13±1.74^{kl}	6.53±1.28^{ghi}	20.23±1.99^{kl}	18.72±2.42^{fgh}

The color of raspberries and berry pulps is a major factor linked to their quality, and preserving the natural color pigments in thermally processed foods poses a significant challenge in food production (Badin et al., 2020). This means that fruits with a lighter red color and less blue (higher Hue°) at harvest also maintain better (lighter) color after storage.

In our study the Brightness (L*) oscillated between 13.72 for '16-22-8' hybrid and 25.15 for '16-23-20' hybrid. The a*-axis (red-green) and b*-axis (yellow-blue) chromaticity have a higher value of 25.17 for '16-23-18' and 10.1 for '16-22-15'.

Chroma Index (C*) oscillated between 15.69 for '16-1-14' hybrid to 26.76 for '16-23-18' hybrid. The angle (h °) oscillated between 17.1 for '16-1-12' hybrid and 26.13 for '16-23-12' hybrid.

Table 3 displays the relationship between fruit quality traits from 2021 to 2023. A notably strong correlation is evident between the shape index and the berry, with a correlation coefficient of (r = 0.357**) (Table 4). This can be attributed to the fact that the weight of the berry is influenced by its size. Furthermore, the correlation matrix reveals a negative correlation between the color indexes (a, b, chroma) and total soluble solids (r = -0.122, and r = 0.524** respectively). The color indexes (a*, b* chroma) show a clear and significant correlation with the shape index (r = 0.466**, r = 0.389** si, r = 0.480). The color indexes, specifically b, also show a distinct and significant correlation with brightness (r = 0.499**) and a* (r = 0.655**).

Table 4. Pearson correlation coefficients for the quality indicators for the studied raspberry genotypes (average 2021-2023)

Pearson Correlation	**Berry weight (g/fruit)**	**Shape index**	**Total soluble solid (^{0}Brix)**	**Brightness (L*)**	**Chromaticity a*- axis (red-green)**	**Chromaticity b*- axis (yellow-blue)**	**Chroma Index (C*)**	**The angle (h °)**
Berry weight (g/fruit)	1	0.357**	-0.202	-0.028	0.103	-0.005	0.088	-0.089
Shape index		1	0.043	0.200	0.466**	0.389**	0.480**	0.163
Total soluble solid (^{0}Brix)			1	0.174	-0.122	-0.021	-0.113	0.041
Brightness (L*)				1	0.199	0.499**	0.269*	0.469**
Chromaticity a*- axis (red-green)					1	0.655**	0.989**	0.076
Chromaticity b*- axis (yellow-blue)						1	0.755**	0.800**
Chroma Index (C*)							1	0.216
The angle (h °)								1

CONCLUSIONS

The weight of berries is significantly affected by the specific genotypes and agro-environmental and meteorological conditions. All the genotypes showed increased berry weight in the three years of the study.

The study's findings indicate that the climate in Pitesti-Mărăcineni is conducive to the commercial cultivation of the raspberry genotypes studied, provided that appropriate agricultural techniques are utilized. This is especially true for the hybrids '16-22-5', '16-23-20', '16-1-21', '16-22-8' which demonstrate superior fruit quality and berry weight.

While the 'Heritage' cv. produces attractive fruits, it is best suited for fresh consumption. Both the 'Heritage' cv. and the mentioned hybrids are of interest for breeding programs due to their potential as a source of genetic variability.

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QUALITY OF SOME STRAWBERRY CULTIVARS IN RELATION WITH CONSUMER PREFERENCES

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Abstract

Strawberries (Fragaria x ananassa) are among the first fruits consumed in Romania, being appreciated for their flavour and special nutritional qualities. In the evaluation of the quality of the strawberry fruit, consumers appreciate the fruit appearance, taste and aroma. The aim of this paper was to analyse fruit quality of some strawberry cultivars in relation with consumer preferences. The research took place out at Small Fruit Department of Research Institute for Fruit Growing, Pitesti on 10 strawberry cultivars with different origin. The data were collected for: fruit weight, length, diameter, firmness, the soluble solids, acidity, five colour indicators, and sensory evaluation. The results showed significant differences between cultivars regarding the shape index, the fruit firmness and the highest value of content in total soluble solids was recorded by 'Vibrant' followed by 'Clery'. Regarding the panel test of the fruits the general score ranged between 5.58 ('Vibrant') to 8.22 ('Matis'). 'Sarom' had the highest weight, dark red fruit colour and balanced taste.

Key words: *Fragaria x ananassa, fruit quality, panel test, cultivars.*

INTRODUCTION

Strawberries are among the most appreciated fruits worldwide due to their exceptional flavour and high levels of bioactive compounds with strong antioxidant properties. Consumers appreciate their distinctive sweet and aromatic notes. Main chemical components contributing to the fruit's flavour include sugars, organic acids, phenolic compounds and various volatile organic copounds (Klee & Tieman, 2018). Strawberry aroma is determined by over 360 volatiles low molecular-weight compounds produced during ripening (Ulrich et al., 2018; Fan et al., 2021). Previous studies have shown significant correlations between these volatile compounds and the intensity of sweetness and flavour in strawberries (Tieman et al., 2012; Klee & Tieman, 2018; Fan et al., 2021) as observed by consumer panels evaluating different strawberry genotypes.
However, the visual impression and texture are also crucial for complex sensory perception. When choosing fresh fruits, shoppers consider colour and appearance as important quality factors (Ragaert et al., 2004; Lewers et al., 2020). Also, sensory quality often limits the shelf life of fresh fruits. (Jacxsens et al., 2002; Jacxsens et al., 2003; Ares et al., 2009). The sensory quality of strawberries relies on both the fruit's sensory characteristics and how consumers perceive them (Costell, 2002; Ares et al., 2009). Therefore, the evaluation of strawberry sensory quality has focused solely on their sensory characteristics (Van der Steen et al., 2002; Pelayo - Zaldivar et al., 2003; Péneau et al., 2007; Kim et al., 2020) using a trained assessor panel.
The aim of this paper was to analyse fruit quality of various strawberry cultivars and to understand consumer preferences for cultivars available to grow in Romania. The goal is to assist growers in selecting the most suitable strawberry cultivars based on marketability, sensory quality, and physicochemical measurements.

MATERIALS AND METHODS

The experiment was conducted during 2021-2023 at Small Fruit Department of Research Institute for Fruit Growing Pitești, Romania on ten strawberry cultivars of different origin: two Romanian ('Premial', 'Sarom'), five French ('Daroyal', 'Darselect', 'Dona', 'Matis', 'Magnum'), one Italian ('Clery'), one

Netherlands ('Elsanta') and one English ('Vibrant'). Three replications, 10 plants in each, were planted at distances of 0.3 × 0.90 m using black plastic mulch. The strawberry fruits were harvested at commercial maturity and the fruits samples were analysed immediately after picking. The indicators studied were recorded on a sample of 20 strawberry fruits. The average fruit weight was determined by weighing of each fruit using HL-400 digital balance. The fruit firmness was determined for each sample with a penetrometer Bareiss HPE II Fff non-destructive test, with a measuring surface of 0.50 cm^2. The length and diameter of the fruit were determined by measuring this using digital calliper. The shape index of the fruit was calculated as the ratio of these two dimensions (Tudor et al. 2014, Jamieson, 2016). The short-conic strawberries have length/width of about 0.9-1.1 and long-conic fruits 1.2-1.4. The soluble solids content was determined with digital refractometer PR Series. The pH values were measured in strawberry fresh juice using a pH meter (ISFET pH Meter, IQ 125, Japan). The external fruit colour was determined with a colorimeter Konica Minolta CR 400, based on system Huntel L*, a*, b* on both sides of the fruit by measuring the lightness or L* (+100 = white, −100 = black), a*(+60 = red, −60 = green), and b*(+60 = yellow, −60 = blue). Chroma index (colour intensity) was determined by the formula $C = (a^{*2} + b^{*2})^{1/2}$ and hue angle of the formula h° = arctangent (b*/a*), where 0° = red-purple, 90° = yellow, 180° = bluish-green and 270° = blue (McGuire, 1992; Lester & Saftner, 2008), Saftner and Lester, 2009). The sensory evaluation of fruits was recorded by open taste panels consisting of researchers and students from Faculty of Horticulture. Whole fruits were presented to members on platters (10 typical fruits of cultivars), to rate attractively and flavour, in points 1 to 5 (in which 5 designates the best performance) according to a questionnaire used by the Romanian breeders. The rating of fruit appearance was based on fruit size and shape, skin colour. The rating of intern characteristics of fruit was based on taste and aroma. The total point value was obtained by summing the scores for appearance and intern characteristics of fruit. The statistical analysis of the data was performed the SPSS 14.0 software and the Duncan comparison test was used to determine the difference between variants, with an error probability of ≤0.05.

RESULTS AND DISCUSSIONS

The study found significant differences in the investigated parameters among different strawberry cultivars. The quality of strawberries is influenced by factors such as their appearance (color intensity, size, and shape), firmness, and aroma. These attributes are affected by a combination of genetic factors, environmental conditions, and cultivation practices. This highlights the importance of considering these factors when evaluating and optimizing the quality of strawberries for commercial production.

The average fruit weight, an important commercial factor for fresh consumption, varied significantly among the cultivars, with 'Sarom' showing the highest average fruit weight of 28.73 g compared to other cultivars (Table 1). During this study, significant differences in fruit shape index were observed, ranging from 0.90 ('Premial') to 1.37 ('Clery'), with the latter exhibiting long-conic fruits (Table 1).

The study found a significant difference in fruit firmness between the cultivars and showed higher resistance value for the 'Dona' (48.49 N) and 'Magnum' (45.07 N) significantly big fruit compared to the other cultivars tested (Table 1).

The flavor of the fruit is influenced by its soluble solids content, as noted by Tomic et al. (2022). The quantitative variation in soluble solids content can be attributed to genetic factors, fruit ripeness, climatic conditions, and various other influences. These factors collectively impact the taste and sweetness of the fruit, emphasizing the importance of understanding and managing these variables for optimizing fruit quality.

The total soluble solids quantity of the studied cultivars ranged from 8.17 to 10.37 °Brix (Table 1), with 'Vibrant' and 'Clery' having the highest values. The pH values were highest for 'Darselect' and 'Vibrant' (4.15) and the lowest for 'Sarom' (3.51).

Table 1. Fruit size, firmness and total soluble solids characteristics and pH of strawberry cultivars (average ± SD; 2021-2023)

Cultivar	Fruit weight (g/fruit)	Fruit shape index	Fruit firmness (N)	Total soluble solids (°Brix)	pH
	19.25±3.60^{b*}	1.37±0.07^{a}	30.8±7.28^{cd}	10.27±0.50^{a}	3.56±0.06^{bc}
'Daroyal'	20.64±1.71^{b}	1.17±0.10^{bc}	31.33±11.87^{c}	8.17±1.16^{d}	3.90±0.08^{ab}
'Darselect'	20.26±2.51^{b}	1.15±0.14^{bc}	33.53±3.46^{bc}	10.37±0.64^{a}	4.15±0.12^{a}
'Dona'	21.37±1.81^{b}	1.28±0.03^{ab}	48.49±3.13^{a}	8.50±0.20^{cd}	3.84±0.41^{abc}
'Elsanta'	18.49±2.50^{b}	1.01±0.03^{cd}	25.00±7.60^{cde}	8.40±0.54^{cd}	3.82±0.23^{abc}
'Matis'	21.12±2.48^{b}	0.97±0.05^{d}	33.23±9.98^{bc}	8.50±0.50^{cd}	4.07±0.23^{a}
'Magnum'	17.55±1.42^{b}	1.18±0.17^{bc}	45.07±9.07^{a}	9.42±0.35^{abc}	3.80±0.14^{abc}
'Premial'	18.2±2.41^{b}	0.90±0.05^{d}	17.90±1.97^{e}	9.17±0.50^{bcd}	3.58±0.13^{bc}
'Sarom'	28.73±4.44^{a}	1.21±0.07^{ab}	26.97±7.35^{cde}	10.03±0.06^{ab}	3.51±0.07^{c}
'Vibrant'	17.4±0.35^{b}	1.15±0.14^{bc}	33.53±3.46^{bc}	10.37±0.64^{a}	4.15±0.12^{a}

*The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($p \leq 0.05$)

The colour of strawberries is a crucial factor as it directly impacts the fruit's commercial value. Consumers generally prefer strawberries the fruit with a vibrant, strong, red colour. The fruit's external colour is influenced by its genotype. The study observed statistically similar values for indicators L*, a*, b* C*, and h° among the different cultivars. Specifically, L* values from 26.09 ('Sarom') to 32.07 ('Elsanta'), C* from 24.06 ('Sarom') to 31.72 ('Elsanta') and h° from 21.8 ('Dona') to 25.38 ('Premial') (Table 2). In this colour spectrum, lower L* and h° values indicate a darker red colour, while the higher values represent the lighter, orange-red colour.

Table 2. Fruit quality colour characteristics of strawberry cultivars (average ± SD; 2021-2023)

Cultivar	Brightness (L*)	Chromaticity a*- axis (red-green)	Chromaticity b*- axis (yellow-blue)	Chroma Index (C*)	The angle (h°)
'Clery'	29.69±2.61^{ab*}	27.49±3.92^{a}	12.08±2.39^{ab}	30.03±4.50^{a}	23.62±1.83^{a}
'Daroyal'	29.31±1.12^{ab}	26.07±0.96^{a}	11.68±1.34^{ab}	28.59±0.63^{a}	24.15±3.07^{a}
'Darselect'	29.43±3.75^{ab}	27.77±5.46^{a}	12.57±2.60^{ab}	30.48±6.03^{a}	24.33±0.66^{a}
'Dona'	29.75±1.37^{ab}	28.64±2.71^{a}	11.42±2.37^{ab}	30.85±3.38^{a}	21.58±2.38^{a}
'Elsanta'	32.07±0.35^{a}	28.77±3.17^{a}	13.31±0.42^{a}	31.72±2.9^{a}	24.97±2.37^{a}
'Matis'	29.92±1.67^{ab}	26.62±4.91^{a}	12.16±1.45^{ab}	29.27±5.07^{a}	24.70±1.36^{a}
'Magnum'	30.19±2.59^{ab}	25.52±0.97^{a}	11.56±1.49^{ab}	28.04±1.08^{a}	24.34±2.92^{a}
'Premial'	26.48±3.63^{b}	27.27±2.15^{a}	12.97±1.82^{a}	30.21±2.54^{a}	25.38±2.36^{a}
'Sarom'	26.09±0.59^{b}	22.17±3.93^{a}	9.26±0.98^{b}	24.06±4.00^{a}	22.77±1.37^{a}
'Vibrant'	29.43±3.75^{ab}	27.77±5.46^{a}	12.57±2.60^{ab}	30.48±6.03^{a}	24.33±0.66^{a}

*The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($p \leq 0.05$)

Sugars and organic acids, along with volatile and aroma compounds, are the primary factors that influence the sensory perception of fruits. These components collectively contribute to the taste, aroma, and overall sensory experience of the fruit. (Milosavljevi´c et al., 2023). Panel evaluations of whole strawberries revealed no significant differences between cultivars in terms of size, form, firmness, visual freshness, glossiness, colour uniformity, and calyx freshness (Table 3).

This suggests that strawberries from all cultivars were perceived as equally fresh, indicating that they are suitable for further evaluation.

The panel also did not detect significant cultivar differences in strawberry size acceptability (Table 3). 'Matis' and 'Sarom' were found to be more acceptable in size (large enough than and 'Darselect' and 'Vibrant' (too small). While 'Sarom' was heavier than 'Vibrant' based on measurable observations, evaluations of form acceptability by the panel were not similar to any physical measurements. The measured length-to-width (l/w) ratio ranged from 0.90 ('Premial') to 1.37 ('Clery') and was not closely associated with the panel's perception of form acceptability, with ranged from 3.48 ('Elsanta') to 4.31 ('Matis').

The assessment of firmness by the panel did not correspond to the texture penetrometer's evaluation of skin toughness. The panel's ratings for firmness were similar to the overall quality. Research by Schwieterman et al. (2014) similarly found that firmer textures are preferred

Table 3. Results of strawberries sensory evaluation (average ± SD; 2021-2023)

Cultivar	Size	Form	Firmness	Color	Calyx freshness	Taste and flavour	General score
'Clery'	3.49±1.10[a*]	3.86±1.21[a]	3.50±0.76[ab]	3.82±1.22[a]	3.32±1.03[a]	3.84±0.89[a]	6.81±2.11[a]
'Daroyal'	3.85±0.48[a]	4.28±0.58[a]	4.59±0.57[a]	3.36±0.79[a]	3.37±0.55[a]	4.06±0.47[a]	7.22±0.99[a]
'Darselect'	3.23±0.59[a]	3.80±0.50[a]	3.30±0.89[ab]	3.58±0.71[a]	3.98±1.34[a]	3.87±0.57[a]	7.21±1.86[a]
'Dona'	3.84±0.79[a]	3.93±0.48[a]	3.06±0.57[b]	3.71±0.56[a]	3.45±0.71[a]	3.97±0.41[a]	7.29±1.47[a]
'Elsanta'	3.4±0.99[a]	3.48±0.84[a]	3.37±0.77[ab]	3.27±0.79[a]	2.97±1.04[a]	3.04±0.44[a]	6.36±2.03[a]
'Matis'	4.37±0.77[a]	4.31±0.86[a]	3.50±0.76[ab]	3.59±0.68[a]	3.86±0.79[a]	3.31±0.63[a]	8.22±1.54[a]
'Magnum'	3.83±0.95[a]	3.74±0.89[a]	4.36±0.67[ab]	4.31±0.86[a]	4.27±0.35[a]	3.91±0.51[a]	8.1±1.27[a]
'Premial'	3.71±1.00[a]	3.79±0.89[a]	3.07±0.59[b]	3.46±0.80[a]	3.48±0.50[a]	3.59±0.30	7.19±1.48[a]
'Sarom'	4.09±0.94[a]	3.79±0.97[a]	4.10±0.88[ab]	3.62±1.00[a]	3.62±1.00[a]	3.88±0.69	7.71±0.87[a]
'Vibrant'	2.88±0.53[a]	3.71±0.75[a]	4.03±0.36[ab]	3.65±0.57[a]	2.69±0.63[a]	3.34±0.18	5.58±0.93[a]

*The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($p \leq 0.05$)

The panel test did not detect significant differences in glossiness and visual colour intensity. The panels rated 'Elsanta' as having the lowest colour intensity score (Table 2), with the highest L* and C* values, indicating that these strawberries were lighter red and had the greatest colour intensity (Table 3). 'Sarom' was among the lowest for the colour parameters, L* and b*, indicating it was among the darkest and most blue (Table 3). The chroma index was also among the lowest (Table 3), suggesting a less shiny appearance compared to the other cultivars.

The panel observed an interaction between the estimated calyx freshness and the overall quality. Some cultivars were found to have higher calyx freshness than others (Table 3). No significant differences in strawberry flavour and taste were detected by the panel (Table 3).

In the panel test, the scores generally ranged between 5.58 to 8.22, , with the highest score found in the 'Matis' followed by 'Sarom' (7.71), 'Dona' (7.29) ,'Daroyal' (7.22) and 'Darselect' (7.21).

According to the panel test, the scores generally ranged between 5.58 to 8.22, with the highest score found in the ‘Matis’ followed by 'Sarom' (7.71), ‘Dona' (7.29), 'Daroyal' (7.22), and 'Darselect' (7.21) Table 3.

CONCLUSIONS

The cultivars 'Matis' and 'Sarom' have been highly rated for their attributes, with 'Sarom' specifically standing out for its size and quality of fruits. This suggests that both cultivars would be well-suited for cultivation in the central region of Romania and other areas with similar environmental conditions. The positive ratings from panel evaluations and instrumental measures indicate that these cultivars have the potential to thrive in these regions and produce high-quality strawberries.

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BIOLOGICAL AND ECONOMIC CHARACTERISTICS OF PLUM CULTIVARS GROWN IN TWO PLUM-PRODUCING REGIONS OF BULGARIA

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Abstract

A comparative study was done on the biological and economic characteristics of plum varieties of Prunus domestica L. grown under agroecological conditions of Lovech (RIMSA – Troyan) and Gabrovo regions (the Plum Experimental Station Dryanovo). The observations were conducted in the period 2019 – 2021 of the varieties such as 'Čačanska lepotica', 'Čačanska najbolja', 'Čačanska rodna', 'Gabrovska', 'Strinava' and 'Stanley', selected for the control. The phenological phases of flowering and harvest maturity of the fruits were determined. Biometric measurements and the chemical composition of fresh plum fruits were made. It was found that the fruits of the large-size varieties such as 'Čačanska lepotica' and 'Čačanska najbolja' had an average weight (41 – 48 g) for the Troyan region, and 42 – 45 g for the region of Dryanovo, which are suitable for fresh consumption. The fruits of the 'Čačanska rodna', 'Strinava', and 'Gabrovska' contain over 20% dry matter in both regions, which makes them suitable for drying and distillation. The studied plum varieties are suitable for growing in the study areas and may be recommended for the expansion of the variety assortment in the establishment of new plum plantations for fresh consumption and processing.

***Key words**: biometric measurements, chemical analysis, phenology, plum, variety.*

INTRODUCTION

The plum is a traditional fruit species in Bulgaria. The biological and economic characteristics of the plum define it as suitable for growing in the mountain and foot-hill regions. Plum plantations in Bulgaria are concentrated in four main plum-producing regions: Central Balkan Mountain (regions of Lovech and Gabrovo), Sredna Gora (regions of Stara Zagora and Plovdiv), Eastern Balkan Mountain (Targovishte and Shumen) and Western Balkan Mountain (Kyustendil and Sofia). These regions have favourable natural and economic conditions, determining the widespread distribution of the plum and are the main raw material base for the production of plum products for consumption, processing, and export to the foreign market (Vitanova et al., 2010; Sotirov et al., 2015).

In recent years, the interest of producers is growing in creating new intensive plantings and introducing new varieties, which are more productive, and tolerant to diseases and pests (Mladenova et al., 2017). According to data from the Ministry of Agriculture of Bulgaria, in 2020-2021 the areas occupied by plum plantations were 9.272 ha. For the same period, of the newly established plantations of fruit stone species, the largest share was occupied by plums (44%), followed by cherries (31%) (Agrostatistics Department, 2022). Market conditions require the offering to producers and consumers of varieties with high fruitfulness, attractive fruit, different purposes of fruit production, and a longer period of fresh fruit (Milatovic et al., 2018). World and Bulgarian selection provides the market with a set of varieties that meet these requirements. The choice of variety must be according to the agro-ecological conditions of the area where it will be grown. According to the selectionists, a thorough study of the impact of soil-climate conditions on the adaptation of the variety is necessary. Specific climatic conditions can have such an impact that the same variety in one country (region) shows high yields, but in another country, it does not show its biological potential (Glowacka & Rozpara, 2017).

In the current climate change, orchards urgently need strategies to adapt to climate change, such as finding varieties and hybrids resistant to

drought and high temperatures and measures to limit areas with fruit tree varieties vulnerable to drought and/or extreme temperatures and introduction of tolerant varieties, as well as new technologies to counteract the negative effects (Cosmulescu & Gruia, 2016).
The present study aims to enrich the knowledge of the biological and economic qualities of introduced and selected plum varieties grown in two typical plum-producing regions.

MATERIALS AND METHODS

The impact of the soil-climate conditions of the two regions on the vegetative and pomological characteristics of several plum varieties was investigated. The experiments were conducted during the period 2019-2021 in two plum plantations in the Lovech and Gabrovo regions, which fall into one of the main plum-producing regions - the Central Balkan Mountain region.
In the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Lovech district), the trees were planted on gray forest soils and at an altitude of 420 m. The plantation of the Experimental Station on Plum in Dryanovo (Gabrovo region) is on pseudopodzolic gray forest soil at an altitude of 300 m. In both plantations, the experimental trees were in full fruit-bearing period, as 5 trees of each variety were studied. The experiments were conducted with the following introduced and selected varieties: 'Čačanska najbolja', 'Čačanska lepotica', 'Čačanska rodna', 'Gabrovska', 'Strinava', and 'Stanley', selected for the control. The trees were grafted on yellow cherry plum (*Prunus cerasifera* Ehrh.) and a planting distance of 5x4 m, with tillage in the inter-row spacing and grown under non-irrigated conditions and the same level of agrotechnics.
During the vegetation of the plum trees, the flowering phases and fruit ripening periods were established for both regions.
At the onset of the technological maturity of the fruits, average samples of 30 fruits were taken for each variety and the following indicators were recorded:
Biometrical
- weight of fruit and stone fruit (g), relative share of fruit stone (%)
- fruit sizes - height, width, and thickness (mm);
- fruit yield (kg/tree).

Biochemical
- Refractometric solids (%);
- sugars by the method of Bartran and Kolthoff (%);
- organic acids by titration with 0.1 NaOH (%);
- tannins according to the method of Leventhal-Neubauer (%).

The observations and reports were conducted according to the methodology for the study of plant resources (Nedev et al., 1979).
The results were statistically processed by the method of analysis of variance (ANOVA) in Microsoft Excel (Data Analysis), and the LSD indicator was used at a significance level of 5% ($P<0.05$).

RESULTS AND DISCUSSIONS

In 2019, the average monthly temperature in March was 7.5ºC for Troyan and 10.07ºC for Dryanovo (Figures 1 and 2). The indicated temperatures are higher than the average characteristic of both regions, which is why the earliest onset of flowering is observed for the study period. In both regions, the beginning of flowering was observed on March 28 - 29 for 'Čačanska lepotica', 'Čačanska rodna', and 'Gabrovska', whereas this phase occurred on 01/04 April for 'Strinava', 'Čačanska najbolja', and 'Stanley'. The beginning of full flowering occurred simultaneously in both regions, during the first ten days of April. The end of flowering for the Dryanovo region occurred three days earlier (April 13 - 18), whereas in the Troyan region, it happened on April 16 - 18 (Figure 3).
The flowering phases in 2020 occurred two days earlier for the Troyan region, compared to the same phases for the Dryanovo region. This is due to the reported higher average monthly temperature in March (7.1ºC) in Troyan, compared to the typical one for the same month of the 20-year base period (6.1ºC) (Figure 1). For this reason, the beginning of flowering occurred in the interval 07 - 12 April. The earliest ripening period was reported for 'Čačanska lepotica' and 'Čačanska rodna', whereas the latest was for 'Čačanska najbolja' (Figure 4).
For the Dryanovo region, the beginning of flowering was observed on April 9, which for most varieties occurred simultaneously, while

for ‘Stanley’ and ‘Čačanska najbolja’ the peak was observed on April 11-12. The end of flowering for ‘Čačanska rodna’ was observed on April 19 (for the Troyan region), whereas in the other varieties, this phase occurred after two to three days. It was the latest for ‘Čačanska najbolja’ on April 26. The end of flowering for the Dryanovo region occurred between April 19 and 27, first with ‘Čačanska lepotica’, ‘Gabrovska’ and ‘Strinava’ varieties and lastly with ‘Čačanska najbolja’ (Figure 3).

The beginning of the vegetation in 2021 occurred later than the previous two years, which is because of the lower average monthly temperatures in March (3.6ºC - Troyan; 4.8ºC - Dryanovo) and April (9.4ºC - Troyan; 9.9ºC - Dryanovo) for both regions, compared to the typical temperatures for the regions (Figures 1 and 2). The beginning of flowering was in the second ten days of April, and in the Troyan region it took place between April 10-15, and in the Dryanovo plantations, it occurred April 13-20. In the period April 27-29, the end of flowering was marked for Troyan, whereas in Dryanovo this phase lasted until May 2 for ‘Čačanska najbolja’.

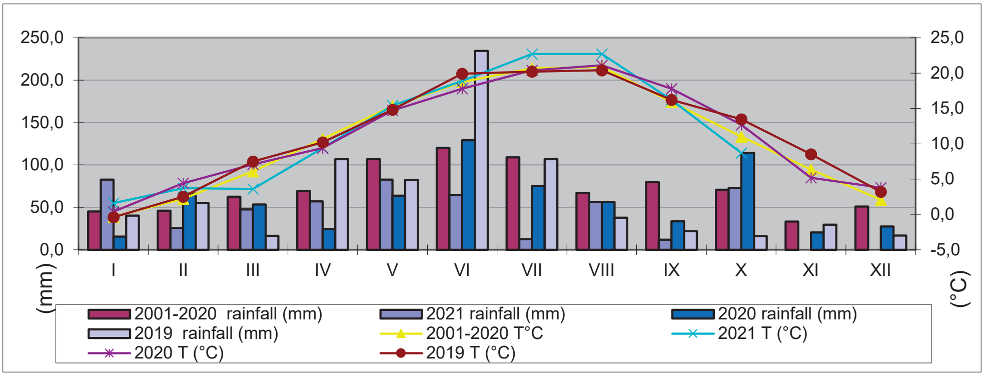

Figure 1. Average monthly temperature and monthly amount of precipitation for 2019-2021 (Troyan)

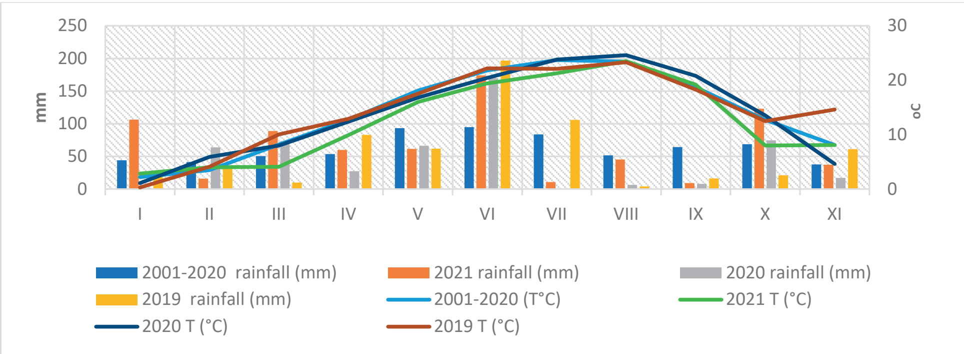

Figure 2. Average monthly temperature and monthly amount of precipitation for 2019-2021 (Dryanovo)

The earliest flowering period in both regions was observed in ‘Čačanska lepotica’, ‘Čačanska rodna’, and ‘Gabrovska’, two to three days earlier than the ‘Stanley’ standard. In the ‘Strinava’ variety, the flowering phases occur at the same time as in the ‘Stanley’ variety. ‘Čačanska najbolja’ began its flowering period up to 3 days later.

For the entire period of the study, the fruits of ‘Čačanska lepotica’ ripened the earliest at the end of July in the Dryanovo region (July 22-29). In the Troyan region, the same variety reached harvesting maturity at the beginning of August (02-09.08.). For the same region, in 2020, the earliest fruit ripening was observed for ‘Čačanska najbolja’ (31.07.), and in the other years, this variety ripened between 16.08

and 18.08. Under the climatic conditions of Dryanovo, the fruits of ‘Čačanska najbolja’ ripened at the same time as those of ‘Stanley’, at the end of August (20-28.09.). Fruits of ‘Gabrovska’, ‘Strinava’, and ‘Čačanska rodna’, ripened in mid-August (10-20.08.) in both regions (Figure 4). The ‘Stanley’ variety was harvested at the latest (20.08-07.09.) in the Troyan region.

Studies conducted under the climate conditions of Serbia show that the fruit ripening of ‘Čačanska najbolja’, ‘Čačanska rodna’, and ‘Gabrovska’ varieties occurred in the first ten days of August, whereas the fruit of ‘Strinava’ ripened in the middle of the month (Milatovic et al., 2018).

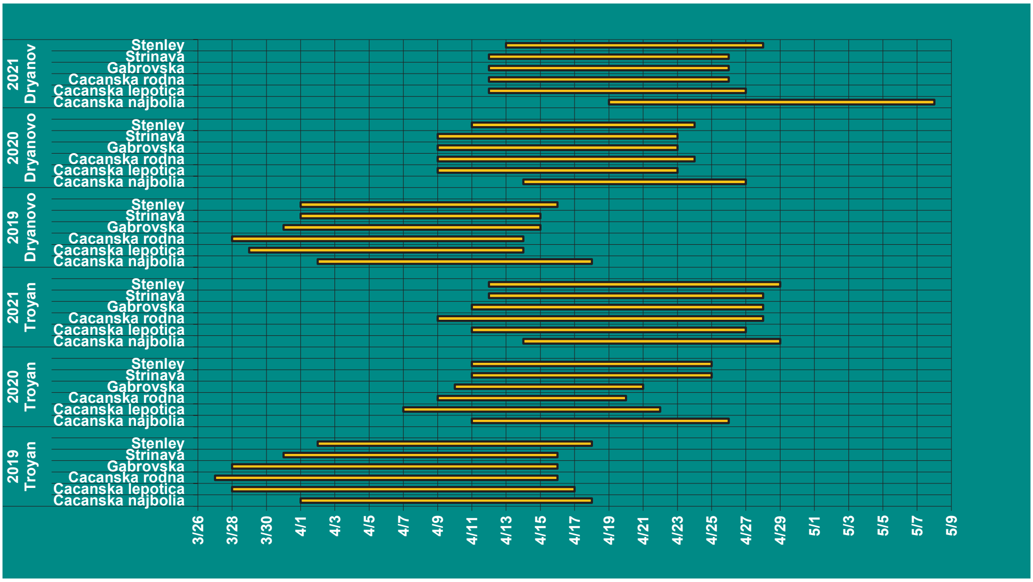

Figure 3. Flowering phases (2019-2021)

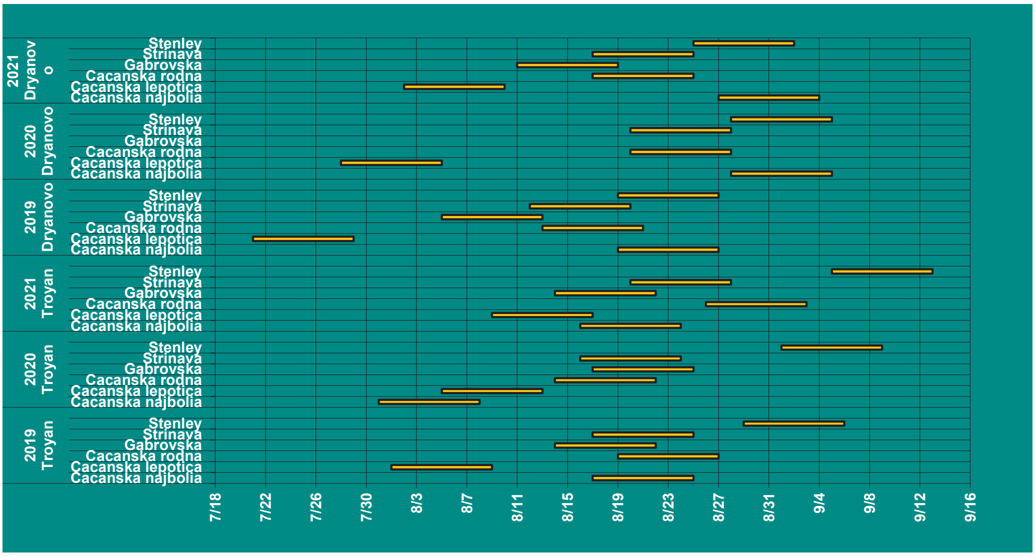

Figure 4. Fruit ripening periods (2019-2021)

The study shows that in 2019 the ‘Čačanska najbolja’ and ‘Čačanska lepotica’ varieties had an average fruit weight of 73.32 - 40.72 g for the Troyan region and 59.11 - 35.69 g for the Dryanovo region. For the rest of the varieties, the average weight was from 27.14 g for ‘Čačanska rodna’ to 31.17 g for ‘Strinava’ in the Dryanovo region, whereas in Troyan the fruits of the ‘Gabrovska’ variety had a weight of 29.88 g, and those of ‘Strinava’ was 38.27 g (Tables 1 and 2).

Table 1. Morphological indicators of fruits from the Troyan region (2019-2021)

Varieties	Fruit weight (g)	Stone weight (g)	Share of stone (%)	Fruit sizes (mm)			
				Length	Width	Thickness	Fruit stalk length (mm)
2019							
Čačanska najbolja	73.32	2.4	3.27	56.14	48.98	45.38	16.60
Čačanska lepotica	40.72	1.6	3.92	43.30	40.29	37.30	11.56
Čačanska rodna	33.25	1.3	3.90	46.08	36.64	33.10	16.61
Gabrovska	29.88	1.1	3.68	43.75	33.76	33.79	13.39
Strinava	38.27	1.2	3.13	46.22	37.70	36.71	15.61
Stanley	43.68	2.3	5.26	49.03	37.12	38.04	17.10
2020							
Čačanska najbolja	48.44	2.5	5.16	47.76	40.90	41.82	13.17
Čačanska lepotica	38.01	1.6	4.20	42.09	38.37	36.51	9.50
Čačanska rodna	31.83	1.3	4.14	44.94	36.49	33.69	15.05
Gabrovska	24.16	1.2	4.96	43.75	33.79	33.76	13.39
Strinava	32.08	1.3	4.05	46.22	36.71	37.70	15.61
Stanley	37.35	2.2	5.89	48.83	36.38	36.43	16.66
2021							
Čačanska najbolja	48.12	2.7	5.61	48.83	42.17	40.18	10.38
Čačanska lepotica	36.02	1.8	4.99	43.42	37.61	37.33	7.62
Čačanska rodna	17.77	1.1	6.19	38.11	30.01	26.73	14.27
Gabrovska	25.40	1.2	4.72	43.85	32.14	32.75	13.23
Strinava	29.14	1.2	4.11	44.37	32.99	34.65	12.39
Stanley	37.56	2.2	5.85	52.15	38.57	35.54	12.85
LSD 0.05	*3.6*	*0.2*	-	*1.6*	*1.2*	*2.8*	*3.1*
LSD 0.01	*4.7*	*0.1*	-	*2.0*	*1.6*	*3.6*	*4.1*
LSD 0.001	*6.0*	*0.1*	-	*2.6*	*2.1*	*4.7*	*5.2*

Table 2. Morphological indicators of fruits from the Dryanovo region (2019-2021)

Varieties	Fruit weight (g)	Stone weight (g)	Share of stone (%)	Fruit sizes (mm)			
				Length	Width	Thickness	Fruit stalk length (mm)
2019							
Čačanska najbolja	59.11	2.0	3.38	49.20	43.82	42.19	16.51
Čačanska lepotica	35.69	1.1	3.08	39.46	36.48	37.53	15.80
Čačanska rodna	27.88	1.4	5.02	42.98	32.08	33.59	21.42
Gabrovska	28.75	0.9	3.13	41.70	33.58	33.39	12.81
Strinava	31.36	1.4	4.46	42.86	33.01	31.17	13.20
Stanley	39.52	1.9	4.80	44.48	36.40	36.17	19.02
2020							
Čačanska najbolja	40.82	2.0	4.89	43.61	38.87	38.19	18.61
Čačanska lepotica	35.51	1.3	3.66	40.58	36.19	36.53	10.84
Čačanska rodna	22.09	1.0	4.52	37.27	30.60	29.07	17.01
Gabrovska	24.16	1.0	4.13	37.78	29.360	28.00	10.78
Strinava	23.97	1.2	5.00	38.00	31.68	33.34	16.38
Stanley	30.54	1.7	5.56	43.41	34.76	33.33	19.05
2021							
Čačanska najbolja	45.14	1.8	3.98	41.03	41.4	40.2	12.90
Čačanska lepotica	42.16	1.4	3.32	43.73.	36.26	38.77	9.86
Čačanska rodna	29.60	0.9	3.04	38.62	31.12	31.75	20.95
Gabrovska	28.61	0.9	3.14	41.73	33.65	33.32	14.04
Strinava	28.34	1.4	4.94	40.08	36.02	33.17	17.87
Stanley	36.47	1.8	4.93	44.85	36.12	36.33	18.78
LSD 0.05	*3.0*	*0.1*	-	*1.6*	*1.5*	*1.8*	*2.8*
LSD 0.01	*4.0*	*0.1*	-	*2.2*	*2.0*	*2.5*	*3.4*
LSD 0.001	*5.1*	*0.2*	-	*2.8*	*2.5*	*3.0*	*4.2*

For the Dryanovo region in 2020, lower values of the morphological indicators of the fruits were recorded than those for the Troyan region. When comparing the fruit weight, the average weight of 'Čačanska najbolja' is 48.44 g, for 'Čačanska rodna' it is 31.75 g in Troyan region. In comparison, fruit weight in the Dryanovo region was 40.84 g for 'Čačanska najbolja' and 22.09 g for 'Čačanska rodna'. The fruit weight of 'Gabrovska' is 24.16 g, for the conditions of both regions.

In 2021, the lowest fruit weight for the entire experimental period was measured for 'Čačanska rodna' variety (17.77 g) in the Troyan region. For the same variety in the Dryanovo region, an average weight of 29.60 g was reported. The 'Čačanska lepotica' and 'Gabrovska' varieties had a smaller fruit weight in Troyan (35.02 and 25.50 g) than values measured in Dryanovo (42.16 and 28.34 g).

There is a variation in the fruit weight of 'Čačanska rodna' in the Troyan region. In 2019 the average weight was 33.25 g and in 2021 it was 17.77 g (Table 1). Similar variation in the weight of the same variety was also found by other authors (Milatovic et al., 2016; Mitrovic et al., 2020). A similar variation for the Dryanovo region was reported for the 'Strinava' variety in 2019. The fruit weight was 31.17 g, and in 2020 was 23.97 g (Table 2).

'Gabrovska' and 'Čačanska rodna' varieties are distinguished by a small fruit stone (its average weight is from 0.9 to 1.1 g), and the largest size was found in the 'Čačanska najbolja' variety (2.0 and 2.7 g) for both regions.

The highest relative share of fruit stone to fruit weight was reported in the 'Stanley' standard (5.85 to 5.56%), in both regions. A higher percentage was reported for 'Strinava' (5.00%), 'Čačanska najbolja' (5.61%) and 'Čačanska rodna' (6.11%) over the years. In 'Gabrovska', 'Čačanska lepotica' varieties, and in some years in 'Strinava', the share of fruit stone did not exceed 4%.

Fruit sizes varied significantly among varieties. The highest value of fruit height was reported for 'Čačanska najbolja' (56.14 mm in Troyan and 49.20 mm in Dryanovo), whereas the lowest value was found in 'Čačanska rodna' (38.11 mm in Troyan and 37.27 mm in Dryanovo). For the rest of the indicators, this trend is maintained for both regions. The longest fruit stalk was measured in 'Čačanska rodna' (15.05 - 21.42 mm), and the shortest in 'Čačanska lepotica' (7.65 mm to 9.86 mm). The remaining varieties have a medium-long stalk

The chemical analysis shows that for the conditions of Troyan, the highest content of dry matter is measured in the 'Strinava' variety (21.50%), whereas it is the lowest (15.56%) in 'Čačanska najbolja' (Table 3). 'Stanley', as a standard variety, is distinguished by the highest content of total sugars (12.33%), compared to the other varieties, which is the lowest in 'Gabrovska' fruits - 7.46%. The 'Stanley' variety has the highest content of inverted sugars (8.74%), whereas the 'Gabrovska' variety has the lowest values (3.63%). The highest content of sucrose is found in 'Čačanska rodna' (4.80%), whereas the lowest value is reported for 'Čačanska lepotica' (1.31%). Low values of organic acids below 1% were reported in all studied varieties in the Troyan region, as they were from 0.54% for 'Strinava' to 0.74% for 'Čačanska najbolja' and 'Čačanska lepotica'. Tannins are below 0.200% in all varieties, as the lowest value is registered in Serbian varieties, compared to the 'Stanley' standard (0.169%). In the Dryanovo region, 'Čačanska rodna' has 22.13% dry matter, followed by Strinava' with 20.22% and the lowest content is in 'Čačanska lepotica' with 17.62% (Table 3). 'Čačanska rodna' has a higher content of total sugars compared to the 'Stanley' standard (11.56%), whereas the other varieties are in this range. The fruits of 'Strinava' have the highest content of inverted sugars (8.10%), and those of the 'Gabrovska' variety have the lowest (5.01%). Serbian varieties have a higher percentage compared to the 'Stanley' standard (7.07%). The highest percentage of sucrose is reported in 'Čačanska rodna' (4.75%), whereas the lowest value is registered again in 'Strinava' (2.83%). In the 'Stanley' standard, the value is 4.30%, and the other varieties have a similar value. The highest value of organic acids is found in the fruits of 'Čačanska lepotica' and 'Strinava' (1.09%), 'Gabrovska' and 'Čačanska najbolja' have less than 1% and the lowest value is registered in the 'Stanley' standard (0.80%). The least amount of tannins is found in the fruits of 'Čačanska najbolja' (0.338%) and 'Čačanska rodna' (0.379%), while for the other varieties, the values approach the 'Stanley' standard (0.459%).

The combined results from both regions indicate that 'Čačanska rodna' and 'Strinava' varieties have more than 20% dry matter, whereas 'Čačanska lepotica', 'Čačanska najbolja', and 'Gabrovska' have a lower percentage than the 'Stanley' standard. The content of organic acids for the Troyan region is below 0.74%, whereas for the Dryanovo region, it is above 0.80%. The 'Gabrovska' variety had the lowest content of total sugars, whereas their content was the highest in the fruits of 'Čačanska lepotica' and 'Čačanska rodna'. The highest value of sucrose is found in 'Čačanska rodna' variety.

Fruits containing a high percentage of dry matter and a low percentage of organic acids are suitable for processing, such as 'Čačanska rodna' and 'Strinava' varieties (Milatovic et al., 2016). Similar results of biometric measurements and chemical analysis of fruits were reported by publications of other authors who studied the same varieties (Bozhkova, 2013; Milatovic et al., 2016; Milatovic et al., 2018).

Table 3. Chemical composition of fresh plum fruits on average for 2019-2021

Varieties	Dry matter (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Tannins (%)	Acids (%)	Gluco acidimetric index
Troyan							
Čačanska najbolja	15.56a	9.61ns	5.71ns	3.70ns	0.142ns	0.74ns	12.72ns
Čačanska lepotica	17.73a	11.51ns	7.33ns	1.31ns	0.130ns	0.70ns	16.20ns
Čačanska rodna	20.50bc	9.83ns	5.63ns	4.08ns	0.142ns	0.61ns	16.11ns
Gabrovska	17.50a	7.46ns	3.85ns	3.05ns	0.163ns	0.63ns	11.77ns
Strinava	21.50c	8.92ns	5.90ns	2.34ns	0.184ns	0.54ns	16.99ns
Stanley	19.36b	12.33ns	8.74ns	3.32ns	0.169ns	0.56ns	23.61ns
LSD 0.05	*2.9*	*4.5*	*4.5*	*2.2*	*0.1*	*0.2*	*8.9*
Dryanovo							
Čačanska najbolja	19.51ab	11.13b	7.44ns	3.73b	0.338nss	0.92ns	12.05ns
Čačanska lepotica	17.62a	10.81ab	7.28ns	3.53ab	0.410ns	1.09ns	9.94ns
Čačanska rodna	22.13c	12.32bc	7.74ns	4.75bc	0.379ns	1.08ns	11.35ns
Gabrovska	17.99a	9.81a	5.96ns	3.84b	0.410ns	0.92ns	11.01ns
Strinava	20.22b	10.94ab	8.10ns	2.83a	0.458ns	1.09ns	10.01ns
Stanley	18.79a	11.56b	7.07ns	4.30b	0.459ns	0.80ns	14.52ns
LSD 0.05	*1.9*	*1.2*	*1.4*	*0.8*	*0.2*	*0.2*	*2.7*

Average values followed by a difference in the letters in one column indicate a statistically significant difference (P<0.05).

In both regions, the highest yield was reported for all studied varieties in 2019. The yield from 'Gabrovska' variety is 53 kg/tree (Dryanovo region) and 25 kg/tree (Troyan region), and it is 42 kg/tree for the 'Stanley' standard (Table 4). In 2020, in the Dryanovo region, the amount of rainfall was 0 mm in July and in August 6.5 mm (Figure 2). Based on this, the yield in Dryanovo is lower than that in Troyan, for all studied varieties. In 2021, 'Čačanska lepotica' (Troyan region) and 'Čačanska najbolja' (Dryanovo region) had good fruitfulness (34 kg per tree). For the Troyan region, the yield of the 'Čačanska rodna' variety has been similar over the years, as it has a low coefficient of variation (11.53%). The remaining varieties have over 20% coefficient of variation, which is why the yield varies over the years (Table 4).

Table 4. Average yield (2019-2021)

Years	2019	2020	2021	*Ave*	*Std*	*CV%*
kg per tree						
Varieties	**Troyan**					
Čačanska najbolja	19	32	10	20.3	11.06	54.39
Čačanska lepotica	22	19	34	25.0	7.93	31.74
Čačanska rodna	23	29	26	26.0	3.00	11.53
Gabrovska	25	18	17	20.0	4.35	21.79
Strinava	18	9	8	11.6	5.50	47.20
Stanley	42	13	26	27.0	14.52	53.79
	Dryanovo					
Čačanska najbolja	24	26	34	28.0	5.29	18.89
Čačanska lepotica	23	6	21	16.6	9.29	55.74
Čačanska rodna	18	26	18	20.6	4.61	22.34
Gabrovska	53	28	20	33.6	17.21	51.13
Strinava	22	11	14	15.6	5.68	36.29
Stanley	42	13	45	33.3	17.67	53.01

CONCLUSIONS

The flowering phase occurred almost simultaneously in both regions. The earliest date was reported for 'Čačanska lepotica' and 'Gabrovska' varieties, whereas 'Čačanska najbolja' was the latest, compared to the 'Stanley' standard. Harvest maturity occurred from the end of July to the beginning of September, which would ensure a long period of marketing of fresh plum fruits.

The climatic conditions of the Central Balkan Mountain region have a positive effect on the biological and economic qualities of the studied varieties. From the biometric and chemical analyses of the plum fruits for both regions, it was established that:

'Čačanska najbolja' and 'Čačanska lepotica' have large fruits that contain a low percentage of organic acids and a high percentage of total sugars, which makes them suitable for fresh consumption;

The fruits of 'Čačanska rodna' and 'Strinava' varieties have a high percentage of dry matter and sucrose, which makes them suitable for processing;

'Gabrovska' has good fruitfulness and its fruits are suitable for fresh consumption and processing.

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ANALYSIS OF THE TOTAL LIPID CONTENT IN THE KERNELS OF SEVERAL TEMPERATE NUT CROPS ACCESSIONS FROM THE GERMPLASM COLLECTIONS OF UCv-SCDP VÂLCEA, ROMANIA

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Abstract

Lipids are an important group of compounds that provide several biological functions such as: energy storage, cell membrane structure, and signaling. This study has the aims to investigate the total lipid content of fruits belonging to temperate nut crops like walnut (Juglans regia L.*), pecan (Carya illinoinensis), and hazelnut (Corylus avellana* L.*) using the extraction method based on different solvents. The analyzed kernels were collected from six walnut accessions ('Valcor', 'Jupâneşti', 'VL 51 B', 'Payne', 'Lara', and 'Franquette'), one pecan hybrid selection (H 21-13 - 2008) and six hazelnut cultivars ('Valcea 22', 'Romavel', 'Ennis', 'Daviana', 'Du Chilly', and 'Purple Filbert'), all from the germplasm collections of Fruit Growing Research and Extension Station (SCDP) Valcea. The FTIR spectroscopy using attenuated total reflectance and suitable variables (absorbance values at certain wavenumbers) of nut oil samples was utilized at frequency regions of 4000-400 cm^{-1}. The colorimetric sulfo-phospho-vanillin method developed by Van Handel (1985) was used in order to determinate the total lipid content. The results obtained after analyzing the nut kernels emphasized inter- and intraspecific variation depending on the genotype and the solvent used.*

Key words*: walnut (Juglans regia* L.*), pecan (Carya illinoinensis), hazelnut (Corylus avellana* L.*), total lipid content, lycopene, β-carotene, ATR-IR spectroscopy*

INTRODUCTION

The edible seeds of tree nut crops are used as a diet complement and are highly consumed worldwide. The production and consumption of these foods have been increasing, and an enormous global market value is forecasted for the next years. In addition to their high lipid content and nutritional value, they provide health benefits to fat metabolism, heart, skin, and brain (Bizera et al., 2019; Vîjan et al., 2023).

Due to their high fat content, there was a belief that foods high in lipids, such as walnuts, hazelnuts, almonds, and olives, shouldn't be consumed frequently. Nonetheless, an abundance of epidemiological and clinical research has demonstrated that fatty fruits and tree nuts are foods that promote health and are now regarded as important food groups and necessary elements of a balanced diet (Widmer et al., 2015). Additionally, research shows an inverse relationship between these foods' intake and the prevalence of coronary heart and cardiovascular diseases, as well as a positive correlation with blood pressure, visceral adiposity, oxidative stress, inflammation, insulin sensitivity, and cancer, among other factors (Banel and Hu, 2009; Guasch-Ferré et al., 2014; Mericli et al., 2017; Massaro et al., 2020).

Having a low glycemic index, the fruits belonging to temperate nut crops have protective properties in accordance with their chemical composition. Beside to water (2-5%), these conventional functional foods contain proteins (10-25%), biogenic amines (melatonin and serotonin), polyunsaturated fatty acids (omega-3 and 6), vitamins (retinol, niacin, thiamine, biotin, and pyridoxine), minerals (potassium, calcium, magnesium, iron, copper, zinc, and manganese), and fiber (Bizera et al., 2019; Giura et al., 2019; USDA Food Data Central, 2024).

Lipids are an important group of compounds that provide several biological functions such as energy storage, cell membrane structure and signaling (Green and Tzagoloff, 1966). Lipids are esters of fatty acids with different alcohols, the products of metabolism, which in plants are found in almost all organs in variable quantities. Some fatty acids have shown an important role in the development of obesity, diabetes, cardiovascular disease, cancer, and mental illness (Alabdulkarim et al., 2012; Julvez et al., 2021). Due to their excellent biocompatibility, lipids are also used as efficient carriers for drug delivery (Yang and Merlin, 2020).

Lipids include various categories of organic substances such as: oils, fats, waxes and phosphatides, which are included in two classes: 1) saponifiable, which are either esters of glycerin with saturated and unsaturated fatty acids (glycerides) and phosphoric acid (phosphoglycerides), or esters of sphingosine with a fatty acid and a phosphoric acid to which a compound is linked with nitrogen (sphingolipids) or esters of primary and secondary alcohols with fatty acids (cerides), 2) unsaponifiable, which are compounds with an isoprenoid structure. The last category includes phytosterols, steroid hormones, carotenoids and fat-soluble vitamins (Popescu et al., 1980).

Due to their chemical heterogeneity, the classification of lipids is more or less arbitrary. However, lipid constituents, regardless of chemical structures, are similar in terms of common solubility characteristics: they are hardly soluble in water, but they dissolve easily in non-polar solvents.

Carotenoids are a group of natural pigments produced by all photosynthetic organisms. Some of these compounds serve as vitamin A precursors, and other are crucial for visual health (lutein) while most carotenoids act as antioxidant molecules in lipid-rich environments (Popescu et al., 1980).

In plant materials, lipid compounds can be found freely, easily separated by extraction with a suitable organic solvent (petroleum ether, hexane, chloroform, dichloromethane, diethyl ether, methanol, etc.), or bound to other compounds, from which they can be separated only after cleavage in the presence of hydrochloric acid. In the absence of a UV-Vis absorption spectrophotometer and a suitable lipid assay standard, the gravimetric method is used to determine the total lipid content, despite the fact that this method is time- and reagent-consuming. The sample is brought to a fine granulation with a grinder, it is refluxed with a solution of hydrochloric acid at low heat (for one hour), the filtered residue is extracted with a suitable solvent in a Soxhlet extractor (for 3-4 hours), the traces of solvent are removed by drying and, finally, by weighing, the mass of extracted fat is determined.

Over the last years, several extraction methods have been developed (Bligh and Dyer, 1959; Gardner et al., 1985; de Boer, 1988; Booij and van den Berg, 1994; Smedes, 1999). The method called liquid - liquid extraction has been used most frequently. Because of the difficulties in the phase separation and the high number of manual operations, the hot Soxhlet extraction is preferred over liquid - liquid extraction (Manirakiza et al., 2001). However, without a complete extraction of the lipid compounds from samples, the results should be interpreted with care.

Due to the great diversity of lipid compounds and its possibility to bind to other molecules, many researchers (Carlson, 1985; Smedes, 1999; Manirakiza et al., 2001; Cequier-Sánchez et al., 2008; Ramírez, 2022) have tested the ability of different solvents and solvent mixtures to totally solubilize the lipids. These studies have shown that solvents used for lipids extraction should have a high solubility for all lipid compounds and be sufficiently polar to remove them from their binding sites with cell membranes, lipoproteins and glycolipids.

This study intends to investigate the carotenoids (lycopene and β-carotene) and the total lipid content of fruits belonging to several nut crops like walnut (*Juglans regia* L.), pecan (*Carya illinoinensis* (Wangenh.) K. Koch) and hazelnut (*Corylus avellana* L.) by using a reliable and economical colorimetric method. Taking into account that neutral lipids (triglycerides, diglycerides, monoglycerides, and sterols) dissolve well in non-polar organic solvents while more polar lipids (free fatty acids, phospholipids, and sphingolipids) dissolve only in relatively polar solvents, the lipid extraction from the fruits belonging to temperate nut crops was performed in different solvents. Moreover, ATR-FTIR spectroscopy provide fingerprinting information about the molecules in the composition of investigated compounds.

MATERIALS AND METHODS

Plant Materials and Sampling

The analyzed kernels were collected from six walnut cultivars ('Lara'-N1, 'Valcor'-N2, 'Jupânești'-N3, 'Payne'-N4, 'VL 51 B'-N5, and 'Franquette'-N6), one pecan hybrid selection (H 21-13-2008, P) and six hazelnut cultivars ('Romavel'-H1, 'Ennis'-H2, 'Vâlcea 22'-H3, 'Daviana'-H4, 'Du Chilly'-H5, and 'Purple Filbert'-H6), all from the germplasm collections of Fruit Growing Research and Extension Station (UCv-SCDP) Vâlcea. 'Valcor', 'Romavel' and 'Vâlcea 22' are cultivars and 'VL 51 B' and H 21-13-2008 are selections issued from UCv-SCDP Vâlcea breeding programs.

The nuts were kept in their shells between the harvest moment and mid-December 2023 in boxes in a ventilated room. Then, the nuts were cracked and the nut kernels (about 100 g) were transformed into a homogeneous mixture with a grinder and were stored at 3-4°C to perform all analyses.

Chemicals and Reagents

The following chemicals and reagents were used: n-hexane, ethanol, acetone, distilled water, petroleum ether, chloroform, vanillin, phosphoric acid, sulfuric acid, and the standards: triolein, lycopene and β-carotene. All chemicals and reagents were purchased from Merck, Darmstadt, Germany.

Extraction Procedures, Biochemical Determinations and Equipment

For the extraction of lipids, samples from one gram of nut kernels powder in 10 mL solvent (chloroform, n-hexane, and petroleum ether) were used. The mixtures were subjected to the following procedures: vortexing for 2 minutes at 3000 rpm, ultrasonication for 30 minutes at 40 kHz, and centrifugation at 6500 rpm for 30 minutes. Next, the extracts were filtered and the lipid determination was made immediately after extraction. Total lipid content was determined using the method proposed by Van Handel, 1985. The results were expressed in mg triolein per 100 g nut kernel powder.

For the quantitative determination of carotenoids, the supernatant obtained by magnetic stirring for 30 minutes at 1500 rpm of 2 g nut kernels powder in 25 ml volumetric mixture of hexane: ethanol: acetone in a 2:1:1 ratio was used, followed by magnetic stirring for another 10 minutes at 1500 rpm of the mixture after adding 10 ml distilled water. After the phase separation, in 10-15 minutes of rest, the volume of the supernatant was measured. Then, the absorbance spectrum of the supernatant was recorded in the 350-550 nm range, using n-hexane as a control. The lycopene and β-carotene contents were calculated, taking into account the amount of nut kernel powder taken in the analysis and the volume of the supernatant obtained after the separation of the phases in accord with Tudor-Radu et al., 2016. The results were expressed in µg lycopene or β-carotene per 100 g nut kernel powder.

The spectral measurements were made with a UV-Vis Perkin-Elmer Lambda25 and an FTIR Jasco 6300 spectrometer. An ATR accessory equipped with a diamond crystal (Pike Technologies) allows the collection of FTIR spectra directly on a sample without any special preparation. The FTIR spectra were recorded in the region of 4000-400 cm^{-1}, with a detector TGS and apodization Cosine. The spectral data were processed with the JASCO Spectra Manager II software. Samples were scanned at 4 cm^{-1} resolution, accumulation: 100 scans. Background reference spectra were recorded using air after every sample to minimize the interference due to carbon dioxide and water vapor in the atmosphere. Between measurements, the ATR crystal was carefully cleaned using pure acetone (Sigma-Aldrich Co.) and then dried with soft tissue (Topală and Tătaru, 2019; Topală et al., 2020).

Data Analysis

All measurements were taken at room temperature (23°C). All analyses were performed in three replicates. Practically, the extracts necessary for the determinations were analyzed in triplicate.

Statistical analysis was performed with the IBM SPSS Statistics 29.0 software package.

Data were reported as mean ± standard deviation.

RESULTS AND DISCUSSIONS

Table 1 show the values for contents of carotenoids (lycopene and β-carotene) and total lipids extracted in different solvents (chloroform, petroleum ether and n-hexane) with indication of the values of mean and standard deviation for the six walnut accessions ('Valcor', 'Jupâneşti', 'VL 51 B', 'Payne', 'Lara', and 'Franquette'), one pecan hybrid selection (H 21-13 - 2008) and six hazelnut cultivars ('Valcea 22', 'Romavel', 'Ennis', 'Daviana', 'Du Chilly', and 'Purple Filbert').

Table 1. The content of lycopene, β-carotene and total lipids in nut kernel powder belonging to several nut crop accessions

Genotype	Lycopene (µg/ 100 g)	β-carotene (µg/ 100 g)	Total lipids (g triolein/100 g)		
			Petroleum ether	n-hexane	Chloroform
Valcor	354.67±4.51[a]	26.67±2.52[c]	55.33±0.45[a]	59.50±0.05[b]	62.91±0.08[d]
Payne	306.99±3.01[d]	45.33±4.04[b]	51.56±0.12[d]	55.15±0.27[d]	60.51±0.01[e]
Jupâneşti	294.01±3.98[e]	28.02±3.01[c]	55.85±0.09[a]	60.86±0.02[a]	66.95±0.02[a]
VL 51 B	316.33±3.51[c]	28.67±3.06[c]	54.27±0.43[b]	58.64±0.03[c]	64.52±0.03[b]
Lara	249.33±2.52[f]	63.33±2.08[a]	54.28±0.34[b]	60.41±0.44[a]	63.39±0.21[c]
Franquette	344.67±5.51[b]	12.03±1.98[d]	52.71±0.36[c]	54.45±0.06[e]	59.23±0.50[f]
H 21-13 - 2008	425.98±1.73	43.67±3.06	54.14±0.03	63.31±0.04	72.54±0.03
Romavel	304.67±5.03[d]	13.67±2.08[ab]	36.38±0.48[e]	41.18±0.15[e]	45.28±0.17[d]
Ennis	307.67±6.03[d]	14.67±2.31[ab]	43.17±0.49[b]	44.96±0.02[c]	46.21±0.03[c]
Daviana	463.04±6.11[b]	12.33±1.53[b]	41.54±0.17[c]	42.51±0.03[d]	45.56±0.12[cd]
Valcea 22	458.33±4.04[b]	14.33±2.52[ab]	38.98±0.02[d]	40.63±0.17[f]	46.07±0.83[c]
Du Chilly	482.33±9.05[a]	15.01±2.01[ab]	41.74±0.04[c]	49.26±0.14[b]	55.02±0.02[b]
Purple Filbert	304.67±5.03[c]	17.33±1.53[a]	52.18±0.02[a]	55.59±0.02[a]	58.17±0.01[a]

Data are presented as mean ± SD (standard deviation).
Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Regarding the content of carotenoids, the lowest level of β-carotene was 12.03 µg/100 g in 'Franquette' (a walnut cultivar) and 12.33 µg/100 g in 'Daviana' (a hazelnut cultivar), while the highest level was 43.67 µg/100 g in H 21-13-2008 (a pecan hybrid selection), respectively 45.33 µg/100 g for 'Payne' and 63.39 µg/100 g for 'Lara' (two walnut cultivars). The lycopene content ranged from 249.33 µg/100 for 'Lara' walnut cultivar to 482.33 µg/100 g for 'Du Chilly' hazelnut cultivar. Similar results were reported by Bolling et al., 2011; Alasalvar and Bolling, 2015; Bizera et al., 2019; Vîjan et al., 2023.

Regarding the total lipids, the best extraction was in chloroform, followed by n-hexane and, finally, petroleum ether. In agreement with the observations of Carlson, 1985; Smedes, 1999; Cequier-Sánchez et al., 2008; Miraliakbari and Shahidi, 2008; Ramírez, 2022, it appears that an efficient extraction of lipids from biological tissues requires a solvent sufficiently polar to remove the lipids from their association with cell membranes and lipoproteins, but sufficiently non-polar to dissolve neutral lipids. Our results indicate chloroform as the suitable solvent for an efficient extraction of lipids from walnut, pecan and hazelnut kernels. Similar results were reported by Carlson, 1985; Smedes, 1999; Cequier-Sánchez et al., 2008; Miraliakbari and Shahidi, 2008; and Ramírez, 2022.

In chloroform, the six walnut accessions they presented values between 59.23 g triolein/100 g ('Franquette') and 66.95 g triolein/100 g ('Jupânești') whereas the six hazelnut cultivars ranged between 45.28 g triolein/100 g ('Romavel') and 58.17 g triolein/100 g ('Purple Filbert'). Iordănescu et al., 2021 highlighted a lipids content between 56.09% and 66.56% for twenty samples of walnuts from three different locations from the west and north-west of Romania. Köksal et al., 2006 reported the values between 56.07% and 68.52% for the total lipid content in seventeen Turkish hazelnut varieties, using n-hexane for six hours in a Soxhlet extractor. The pecan hybrid selection (H 21-13-2008) showed the highest value for total lipid content (72.54 g triolein/100 g) among the analyzed nuts and recommends the pecan kernels in the human diet. This result is consistent to those published by Rudolph et al., 1992; Wakeling et al., 2001; Miraliakbari and Shahidi, 2008, but higher than those reported by Amaral et al., 2005 and Ribeiro et al., 2020.

Differences of the total lipid content in the fruits belonging to temperate nut crops could be attributed to many factors, such as cultivar, crop location, climate, year of production, soil composition, harvest time and extraction methods (Rudolph et al., 1992; Parcerisa, 1993; Ribeiro et al., 2020; Goodarzi et al., 2023).

The main characteristic vibrations in the ATR FTIR spectra are presented in Figures 1-3 for samples N1 ('Valcor'), P (pecan selection) and H1 ('Romavel'). Figures 4 and 5 show the overlapped spectra of walnut and hazelnut samples.

The 400-4000 cm^{-1} region of FTIR spectra shows several characteristic peaks (bond oscillations) at certain wavenumbers that are assigned to specific functional groups of their respective components (Tables 2 and 3).

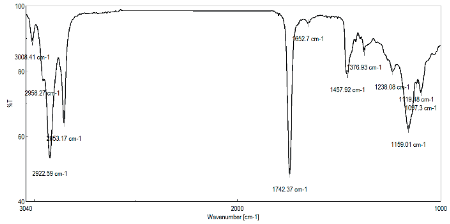

Figure 1. ATR-FTIR Spectrum for 'Valcor' (N1) walnut cultivar sample - chloroform extract

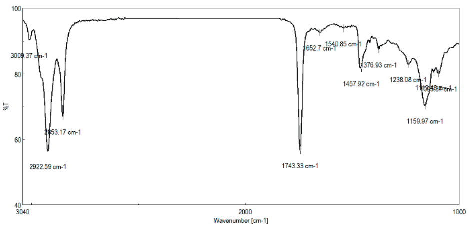

Figure 2. ATR-FTIR Spectrum for pecan hybrid selection (P) sample - chloroform extract

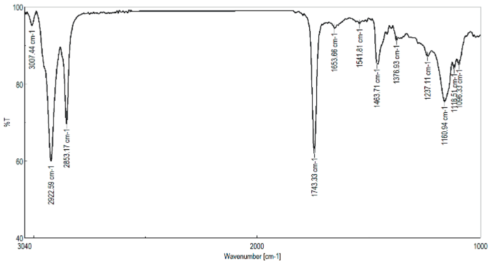

Figure 3. ATR-FTIR Spectrum for 'Romavel' (H1) hazelnut cultivar sample – chloroform extract

The unsaturated C atom of olefins in nuts exhibits C–H stretching vibrations, which peak at 3008–3010 cm^{-1}.

The peaks at 2922 and 2853 cm^{-1} are assigned to asymmetric stretching vibrations of methylene (–CH_2), while peak at 2953–2956 cm^{-1} is attributed to symmetric stretching vibration of methyl (–CH_3) (Topală et al., 2020).

In the region of 1800-1000 cm^{-1} are observed protein amide C=O stretching and amide N–H bending vibrations (60%) coupled to C–N stretching vibration (40%) mode of the polypeptide and protein backbone, respectively. In the same region, the absorption band at 1746 cm^{-1} is due to C=O stretching vibration of ester groups in triacylglycerols and two bands at 1462 cm^{-1} and 1378 cm^{-1} corresponding to the CH absorption bending vibration of CH_2 and CH_3 groups, respectively (Che Man and Setiowaty, 1999; Guillén and Cabo, 1999), are also lipid-related bands.

The bands at 1462 cm^{-1} are correlated to the content of fatty acid chains (Melin et al., 2000).

Table 2. The location of the maxima of the absorption bands FTIR in the tested nuts accessions samples

Attribution	Samples						
	N1	N2	N3	N4	N5	N6	P
C-H ring vas (=C-H), lipids, fatty acids	3008	3008	3008	3008	3009	3010	3009
asymmetric stretching vibration of CH_3	2956	2953	2956	2953	2953	2956	2953
asymmetric stretching vibration of CH_2	2922	2922	2921	2922	2922	2921	2922
symmetric stretching vibration of CH_2	2853	2853	2853	2853	2853	2854	2853
C=O stretching vibration of ester groups	1742	1742	1742	1742	1742	1746	1743
Amide I absorption (predominantly the C=O stretching)	1652	1652	1652	1652	1652	1646	1652
Protein amide II absorption (δN-H, νC-N)	1540	1540	1540	1540	1540	1549	1540
CH_3 bending vibration (lipids and proteins)	1457	1456	1457	1456	1456	1456	1457
vibration of CH_2 and CH_3 groups	1376	1376	1376	1376	1375	1376	1376
Amide III	1238	1237	1237	1238	1238	1238	1238
νC-O of proteins and carbohydrates	1159	1159	1159	1159	1158	1159	1159
Symmetric stretching P-O-C; C-O stretching vibration of C-OH	1119						1119
Stretching PO_2^{2-} symmetric	1097	1098	1098	1098	1098	1098	1095

Codifications: N1–N6 for six walnut cultivars ('Lara'-N1, 'Valcor'-N2, 'Jupânești'-N3, 'Payne'-N4, 'VL 51 B'-N5, and 'Franquette'-N6), and P for one pecan hybrid selection (H 21-13-2008)

Table 2. The location of the maxima of the absorption bands FTIR of the tested hazelnuts samples

Attribution	Samples					
	H1	H2	H3	H4	H5	H6
C-H ring vas (=C-H), lipids, fatty acids	3007	3007	3004	3004	3006	3006
asymmetric stretching vibration of CH_3	2953	2953	2956	2953	2952	2956
asymmetric stretching vibration of CH_2	2922	2922	2921	2922	2922	2922
symmetric stretching vibration of CH_2	2853	2853	2852	2852	2852	2853
C=O stretching vibration of ester groups	1743	1743	1743	1743	1743	1743
Amide I absorption (predominantly the C=O stretching)	1653	1652	1652	1652	1648	1651
Protein amide II absorption (δN-H, νC-N)	1541	1540	1541	1540	1540	1540
CH_3 bending vibration (lipids and proteins)	1463	1463	1456	1456	1456	1456
vibration of CH_2 and CH_3 groups	1376	1375	1376	1376	1376	1375
Amide III	1237	1238	1237	1236	1236	1237
νC-O of proteins and carbohydrates	1160	1160	1160	1159	1159	1160
Symmetric stretching P-O-C; C-O stretching vibration of C-OH	1118	1118	1117	1118	1118	1118
Stretching PO_2^{2-} symmetric	1096	1096	1095	1095	1095	1095

Codifications: H1–H6 for six hazelnut cultivars: 'Romavel'-H1, 'Ennis'-H2, 'Vâlcea 22'-H3, 'Daviana'-H4, 'Du Chilly'-H5, and 'Purple Filbert'-H6

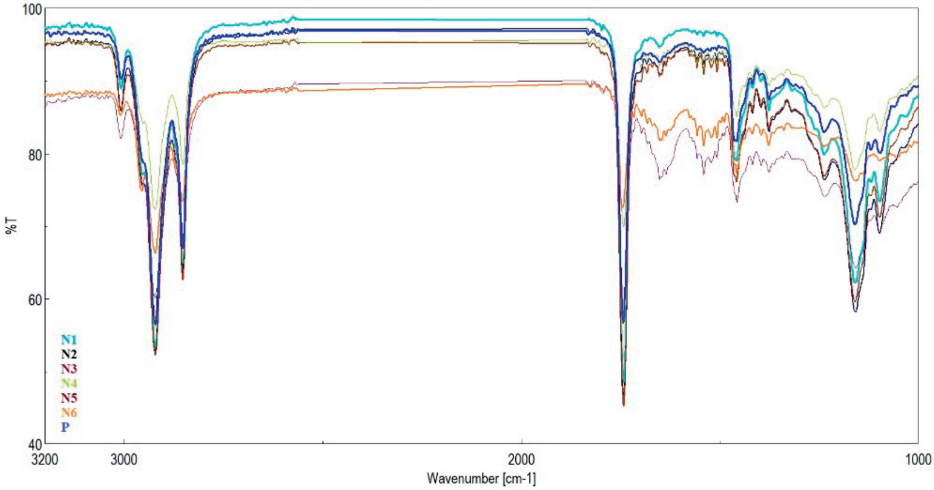

Figure 4. ATR-FTIR spectra of overlapped walnut samples (chloroform extracts)

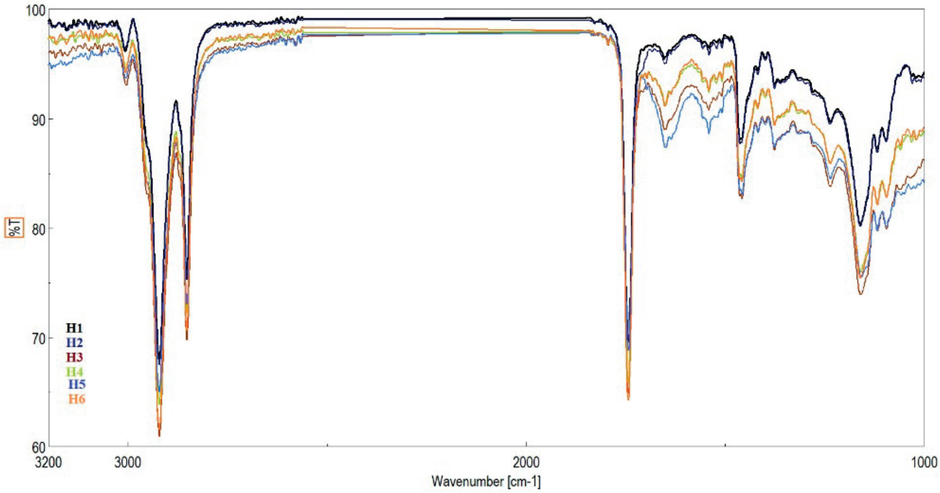

Figure 5. ATR-FTIR spectra of overlapped hazelnut samples (chloroform extracts)

CONCLUSIONS

In the present research, a reliable and economical colorimetric method has been developed for quantitative analysis of total lipid. There are multiple advantages of the modified Van Handel method, such as: 1) the reagents used in analysis are inexpensive and easy to handle, 2) the amount of sample required for the determinations is small and it can be adjusted to fit in the standard range of concentrations of the spectrophotometric analysis, 3) a large number of samples can be analyzed in a short period of time (about two hours), with less labor for the samples preparation and analysis, and 4) the color development of the mixture of lipids with the sulfo-phospho-vanillin reagent is rapidly and consistent.

Regarding the total lipids, the best extraction was in chloroform, followed by n-hexane and, finally, petroleum ether. The pecan hybrid selection (H 21-13 - 2008) presented the highest lipid content (72.54 g triolein/100 g in chloroform), followed by the walnut samples (59.23-66.95 g triolein/100 g in chloroform) and finally the hazelnut samples, with values between 45.28 and 58.17 g triolein/100 g in chloroform. Moreover, the lipid fingerprint was highlighted by FTIR spectroscopy.

As regards to the carotenoid content of the analyzed samples, there is no differentiation between the three plant materials in terms of lycopene content. However, with the exception of the walnut sample from 'Franquette', one can notice that the walnut samples had 1.5 to 3.7 times higher values for the β-carotene content compared to the hazelnut samples.

Nowadays, in order to guarantee the quality and safety of food and agricultural goods, it is crucial to obtain important information on structural elements.

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FROST EVENTS FORECAST USING MACHINE LEARNING IN BULGARIA

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Abstract

In the present study a scheme for damaging frost occurrence forecast in Bulgaria is presented. It is based on Random Forest technique and uses the regional numerical weather prediction (NWP) model ALADIN output as predictor. Initially, the statistical model is trained with measured data with three-hours frequency at 5 representative meteorological stations in Bulgaria during April and May for the period 1991-2020. Using parameters from the regional NWP model production as predictors gives possibility to forecast frost probability 72 hours ahead. The performance of the scheme is evaluated. Results for 27 synoptic stations during April (2021-2023) show a probability of detection above 0.85 and a false alarm rate below 0.1 independently of the remoteness of the forecast. Most of the considered cases were correctly discriminated by the scheme as "frost" and "non-frost" cases, which would not be the case if only considering the forecasted minimum temperature. Our results show that frost could be forecasted by the presented scheme 3 days before its occurrence, which should be enough to react to minimize damage caused in the agricultural sector.

***Key words**: frost, forecast, Random Forest, Bulgaria.*

INTRODUCTION

Increasing tendencies of the average monthly air temperatures in Bulgaria during the last decades are established. The indicated trends of changing temperature conditions are unfavourable for the fruit species, especially the early flowering drupe species. Warming in winter, and especially in January and February, cause premature development of fruit trees and subsequent frosts cause damage to varying degrees, depending on their development. Damage from late spring frosts is a major limiting factor for fruit production in Bulgaria. Frost events are locale and are difficult to be predicted. They are associated, among other factors, with the type of soil, terrain orientation, and the damage caused to plants depends on its intensity and duration. There are several techniques to minimize damage caused by frost in the agricultural sector (De Melo-Abreu et al., 2016), however, it is necessary to know in advance the likelihood for occurrence of this phenomenon. Many warning systems for frost have been developed based on predictions from numerical weather prediction (NWP) models (Prabha and Hoogenboom 2008), vegetation indices estimated by satellites (Gabbrielli et al., 2022), statistical models (Lee et al., 2016), fuzzy logic (Cadenas et al., 2020), neural networks (Fuentes et al., 2018; Ding et al., 2020) and statistical indices (Anandhi et al., 2013; Rozante et al., 2019). In recent years, significant advancements have been made regarding the application of machine learning in studies related to frost prediction (Diedrichs et al., 2018, Jamei et al., 2015, Kalaiarasi and Maheswari 2020, Diniz et al., 2021, Ismail et al., 2021, Talsma et al., 2022, Rozante et al., 2023 and others).

In the present study the scheme for damaging frost events probability forecast in Bulgaria is presented. The scheme is based on Machine Learning and more precisely Random Forest and the operational NWP model ALADIN (Termonia et al., 2018) output as statistical model predictors. A Random Forest Algorithm is an extremely popular supervised machine learning algorithm that is used for Classification and Regression problems based on the concept of ensemble learning which is a process of combining multiple classifiers to solve a complex problem and improve the performance of the model. All statistical analyses for the present study were performed using R Statistical Software (R Core Team 2021)

METHODOLOGY

Every 3-hours measured data for the months of April and May for a period of 30 years (1991-2020) at 5 meteorological stations in Bulgaria are used to train and to test the statistical model. The stations Kjustendil, Stara Zagora, Kneja, General Toshevo and Dobrich (marked in red in Figure 1) are chosen as being of the most representative for occurrence of damaging frost on orchards causing severe loss on national fruit production during the last decades. The following parameters were determined by the model as damaging frost predictors: air and dew point temperature and relative humidity at 2 m, mean sea level pressure, wind speed at 10 m and the hour of measurement. In the statistical model, cases with frost were determined in the following way: for dates when damaging frost was registered at the stations (frost days), which usually is during the night time, the periods between 9 UTC at the previous day until 6 UTC of the frost day are considered as "frost related cases" (and are designated by 1, while "non-frost related cases" by 0 in the model). The model performance for the test sample of data is relatively high with a Probability of Detection (POD) of 0.74 and a False Alarm Rate (FAR) of 0.03.

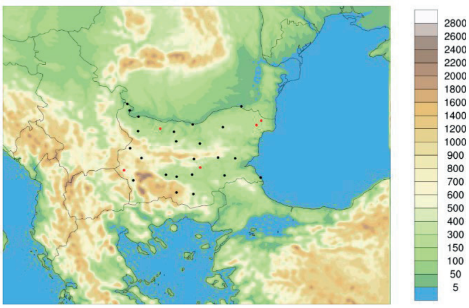

Figure 1. Meteorological stations in Bulgaria from which measurements are used for the present study

Further, for predicting the damaging frost probability, the same parameters are used but taken from the operational NWP model ALADIN. The operational model configuration at NIMH is the following: the integration domain (shown in Figure 1) covers a big part of the Balkan Peninsula, centered on Bulgaria, with a horizontal resolution of 5 km, 105 vertical levels, a time step of 300 s and a forecast range of 72 h. It is run four times daily, at 00, 06, 12 and 18 UTC and it uses the global ARPEGE of Météo-France. To reinforce the impact of night hours forecast (when frost occur) but to take into account also the day hours forecast, the scheme for damaging frost probability was formed in the following subjective way:

- if at least one of the night periods (between 21 and 06 UTC) is determined as a "frost related case" and at least one of the previous day daily periods (between 09 and 18 UTC) is determined as a "frost related case" then the probability of damaging frost occurrence for the day FP =100%;
- if at least one of the night periods (between 21 and 06 UTC) is determined as a "frost related case" and none of the previous day daily periods (between 09 and 18 UTC) is determined as a "frost related case" then the probability of damaging frost occurrence for the day FP = 80%;
- if none of the night periods (between 21 and 06 UTC) is determined as a "frost related case" and at least one of the previous day daily periods (between 09 and 18 UTC) is determined as a "frost related case" then the probability of damaging frost occurrence for the day FP = 50%;
- if none of the night periods (between 21 and 06 UTC) is determined as a "frost related case" and none of the previous day daily periods (between 09 and 18 UTC) is determined as a "frost related case" then the probability of damaging frost occurrence for the day FP = 0%.

RESULTS

Forecast data for the month of April for the 3 years period 2021-2023 were evaluated and verified using the data for damaging frost registered at 27 meteorological stations in Bulgaria shown in Figure 1. For this considered period 133 cases of frost were registered at the considered stations, which are about 10 times less than the "non frost" cases (1296). First, if considering if any damaging frost probability was forecasted (fp>0), Table 1 shows the performance of the scheme for damaging frost detection over the 27 considered synoptic stations. As ALADIN forecast range is 72 h, we have a prediction for 3 days ahead. 1st forecast designates the closest, while 3rd – the farthest. For this study only model run at 06 UTC is considered. It is visible that POD is high, but decreases slightly with the forecast moving away in time (from 0.89 to 0.85 for the 3rd forecast), while FAR is very low, but slightly higher for 1st forecast.

Table 1. April 2021-2023: Probability of Detection (POD) and False Alarm Rate (FAR) of the scheme for damaging frost probability forecast for 1, 2 and 3 days ahead (respectively 1st forecast, 2nd forecast and 3rd forecast)

	1st forecast	2nd forecast	3rd forecast
POD	0.89	0.89	0.86
FAR	0.069	0.066	0.066

From all 133 frost cases, 101 cases (about 76%) were correctly forecasted by all 3 forecasts, while only 4 cases (about 3%) were not predicted by none of the forecasts. 20 cases (about 15%) were correctly forecasted by two of the three forecasts (10 cases are with correct 1st and 2nd forecasts, 4 - with correct 1st and 3rd forecasts, and 6 - with correct 2nd and 3rd forecasts) and 8 cases (about 6%) were correctly forecasted only by one forecast (3 cases with 1st correct forecast, 2 - with 2nd correct forecast and 3 - with 3rd correct forecast).

If considering the determined frost probability FP by the scheme, for about 62% of cases with correct 1st forecast, FP=80% and 38% of these cases are with FP=100%. The cases with correct 2nd forecast were 70% with FP=80% and 30% with FP=100%. 78% of cases with correct 3rd forecast are with FP=80%, while 22% - with FP=100%. There is only one case with FP=50% which in fact was a non-frost case, thus was treated as a false alarm.

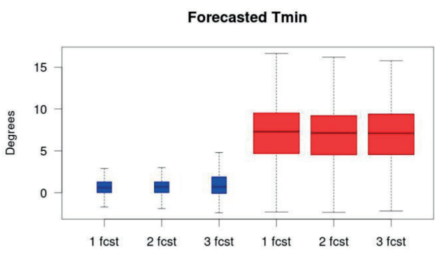

Figure 2. Boxplot of predicted minimum temperature Tmin by 1st, 2nd and 3rd forecasts for cases with (in blue) and without (in red) damaging frost registered

Also, it has to be mentioned that from all 133 frost cases, only for 33 the forecasted minimum temperature is below 0°C. Figure 2 shows the interval of values of the forecasted minimum temperature for all considered cases. As expected, the median of the forecasted Tmin. for frost cases is considerably lower than this for non-frost cases (about 0.6°C for the 3 forecasts for "frost cases" and about 7.1°C for the 3 forecasts for "non-frost cases"). The maximum value of Tmin. for frost cases reaches values above 5°C, while for some non-frost cases Tmin. is below -3°C. Our results show that the majority of these cases were correctly discriminated by our scheme as "frost" and "non-frost" cases, which would not be the case if only considering the forecasted Tmin.

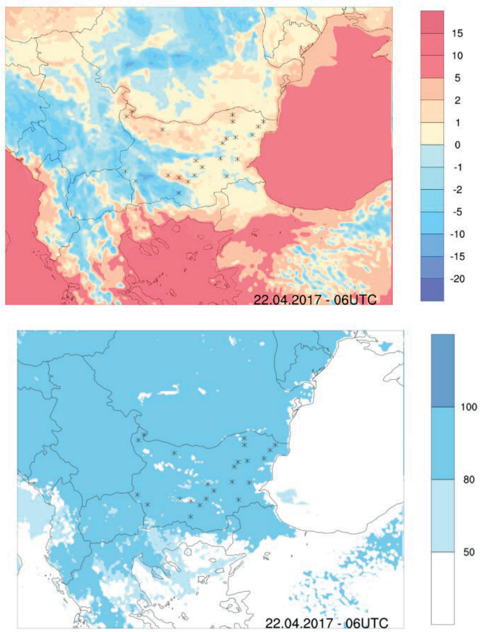

Figure 3. Tmin. forecasted by ALADIN (top panel) and frost probability forecasted by our scheme (bottom panel) for 22/04/2017. Stations with registered damaging frost are indicated with black crosses

Figures 3 and 4 show two case studies with two different frost types. During 22/04/2017 advection frost occurred and was registered at many synoptic stations in Bulgaria. In Figure 3 is shown the forecasted by ALADIN minimum temperature, which is visibly above 0°C over a big part of Bulgaria. However, the scheme for frost probability forecast gives a probability of 80% over almost whole country, covering all stations with detected frost. During 11/05/2023 a considerably late radiation damaging frost (which is very local and more difficult to be predicted) was registered at two synoptic stations – Dobrich and Rojen. In Figure 3 it is visible that the presented here scheme predicts very correctly the probability of frost occurrence.

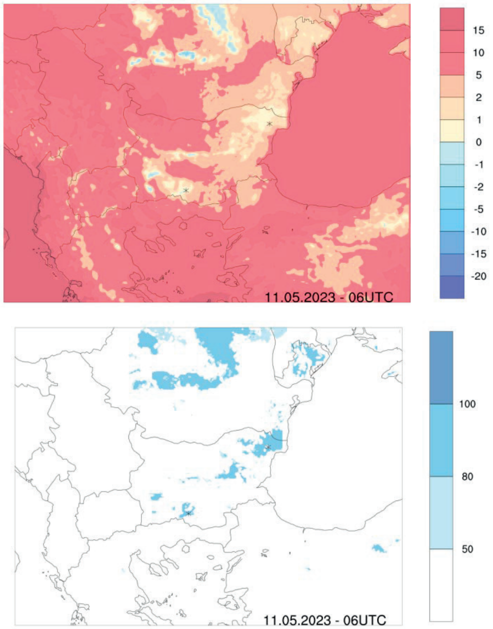

Figure 4. Tmin. forecasted by ALADIN (top panel) and frost probability forecasted by our scheme (bottom panel) for 11/05/2023. Stations with registered damaging frost are indicated with black crosses

CONCLUSIONS

Frosts are a dangerous phenomenon of a meteorological nature, which limits the potential growing season for field and vegetable crops and orchards. Late spring frost often cause annual damage to varying degrees, and during some years, the harvest is totally compromised in some places due to them. This is also related to the tendencies towards an increase in the average monthly temperatures in winter and the earlier start of the vegetation processes. The average multi-year data on the occurrence of the sensitive phases in orchards (Geogieva et al., 2023) are after the average multi-year date of the onset of spring frost, which makes the production of early flowering species risky in many regions of Bulgaria. Timely and accurate specialized forecasting of freezing conditions is the most effective way to limit the consequences of extreme weather events.

In the present study a scheme for damaging frost occurrence forecast based on Random Forest technique and using NWP model output as predictors is presented and its performance is evaluated. Results for 27 synoptic stations during April the last 3 years show a probability of detection above 0.85 and a false alarm rate below 0.1 independently of the remoteness of the forecast. Most “frost cases” were predicted with a frost probability FP=80%, while about a third of them with FP=100%. Our results show that most of the considered cases were correctly discriminated by the scheme as “frost” and “non-frost” cases, which would not be the case if only considering the forecasted Tmin. As there is no significant difference between the closest and the farthest forecast performance, it could be concluded that damaging frost could be relatively well forecasted by the presented here scheme 72 hours (3 days) before its occurrence, which should be enough to react to minimize damage caused by frost in the agricultural sector. Future investigations will be performed to test the forecast performance of the scheme for 10 days ahead. For this aim output from IFS (the model of ECMWF) will be used as statistical model predictor. The presented here scheme will be incorporated in the operational suite as a specialised NWP model post-processing for predicting damaging frost probability occurrence.

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RESEARCH REGARDING THE EFFECT OF IRRIGATION AND FERTILIZATION ON PHOTOSYNTHETIC RATE AT DIFFERENT PLUM CULTIVARS IN THE FRUIT TREES NURSERY, IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

This paper aimed to present the effect of irrigation and fertilization on photosynthetic rate at trees of different plum cultivars in the fruit trees nursery, to obtain a good quality planting material, given the fact that in recent years Romania is facing the phenomenon of complex drought, which represents a climatic hazard phenomenon that induces the most serious consequences in horticulture. The research was carried out in the year 2022 in a fruit trees nursery located in the North- West Region of Romania. The initial biological material was represented by rootstock seedlings that belong to the "Certificate" biological category and for grafting were used buds from two plum cultivars: 'Stanley' and 'Cacanska Lepotica'. During the analysed period, trees of 'Cacanska Lepotica' utilized the fertilization with 16 and 24 kg of NPK at a significantly higher level, with an increases of photosynthesis rate by 7.55%-14.29%. In the case of 'Stanley', the treatment with NPK was associated with a significant intensification of the photosynthesis process compared to the unfertilized variant and smaller variations between fertilization doses.

Key words: *fertilization, irrigation, photosynthetic rate, plum cultivars.*

INTRODUCTION

In the climate context of recent years, Romania is facing the phenomenon of complex agricultural drought, which represents a climate hazard phenomenon that induces the most serious consequences in agriculture. Drought is a prolonged dry period in the natural climate cycle, a slow-onset disaster. In this context, agriculture cannot be sustained without irrigation. Irrigation improves crop growth and quality, allowing farmers to farm on a consistent schedule, creating more reliable and quality food supplies. In the nursery sector, irrigation offers the possibility of obtaining vigorous, viable, quality, healthy, damage-free, disease- and pest-resistant planting material. In addition to water, another essential factor in obtaining a quality fruit tree planting material is the provision of the necessary fertilizers.

Water management and fertilizer applied nutrients are the two major factors affecting growth and productivity in the fruit tree nursery. Sustainable water and fertilizer use in the nursery has become a priority, with the adoption of management strategies that maintain satisfactory yields, thus improving both fertilizer and water use efficiency. In recent years, fruit trees have a very high demand on the market, and the fruits obtained are intended to provide an effective source of vitamins. Nursery stock also has a water requirement, requiring irrigation throughout the growing seasons (Schmid, 2021). In recent years, nurseries have experienced a difficult production period due to factors related to weather conditions.

The economy is mainly dependent on agriculture and climate change can have dramatic effects on it. Agricultural production is low due to the excessive fragmentation of properties but also to the reduction in the degree of mechanization of works, irrigation, and chemical treatment. Plants have adapted to specific environmental conditions, which allow them to carry out their vital processes in optimal conditions and ensure the perpetuation of the species (Schubert, 2022).

Climate changes also affect the rainfall regime: the volume of annual precipitation, their monthly distribution and favour the appearance of dry periods or, on the contrary, periods with excessive precipitation (Rickmann, 2014). Global warming intensifies the process of plant transpiration and water absorption, intensifies the process of water evaporation from the soil surface and reduces the amount of water available for plants (Polak, 2018). High temperatures, strong insolation, drought, and excessive irrigation also increase soil salinity, which has negative effects on plants (Mayer, 2019). The simultaneous action of these stress factors induces numerous morphological, physiological, biochemical, and molecular changes in crop plants, which have unfavourable effects on their growth, development, and production (Goyal, 2021). Like other branches of agricultural production, modern fruit trees growing cannot be conceived without ensuring a water regime corresponding to the requirements of the cultivated species and the culture system used (Miller, 2018). Through the strong root system that makes it possible to explore a large volume of soil and the increased absorption capacity of the roots, many of the fruit tree species ensure the achievement of favourable results even in areas with a lower pluviometric regime or when plantations are located on sloping land and on dry sands, where water is retained more difficult (Pinske, 2017). Being, however, plants with increased specific water consumption, for the development of the growth and fruiting processes at the appropriate level, in the crop areas where the periods of drought have a relatively constant frequency and with extensions over wider time intervals, completing the water deficit through irrigation in fruit trees plantations becomes a necessary, if not indispensable measure (Smith, 2022).

Our country, due to its geographical location at the confluence of the continental and Mediterranean climates, generally offers favourable climate and soil conditions for many fruit trees nurseries. Initially, the fruit trees nurseries were concentrated in the areas with a richer rainfall regime, so that the rootstock capture depended to a greater extent on the rainfall regime, the human intervention at the beginning being modest in this regard.

A characteristic shortcoming of the climatic regime of our country, which is reflected quite significantly in fruit growing, is the defective distribution of precipitation during the year, resulting in prolonged periods of drought in some areas (periods of time longer than 10 days during the vegetation and 14 days during the rest period, in which no rains greater than 5 mm fall). Considering these aspects, associated with the tendency to develop important fruit-growing centres in typically dry areas, on zonal soils and on sands, irrigation must be a concern of prime importance for the fruit trees -growing sector in our country, but which must to manifest differently, depending on the pedoclimatic zone, type of rootstock, etc. (Venig et al., 2022).

Irrigation of fruit trees is necessary in fruit growing, where annual precipitation is below 500 mm, and in areas with precipitation between 500-700 mm/year additional irrigation is applied (Venig, 2006).

With fruit trees in the nursery, as with all cultivated plants, the growth process depends to the greatest extent on the climate and soil conditions available to them (Stănică & Peticilă, 2012). Of these, along with heat, light, air and mineral substances, water plays a very important role. It enters the composition of the various organs of the tree in the proportion of 75-85% and sometimes even more, of their total weight. In addition to the fact that water ensures the circulation of fertilizing elements from the soil to the plant (as well as in the entire plant), water participates as a basic element in the synthesis of all the organic substances that make up the tissues of the rootstocks, respectively of the trees. That is why it is necessary for the trees to always have water available, in sufficient quantity, so that the growth processes take place with as much intensity as possible (Faulkner, 2022).

The fertilization system in the nursery includes long-term activities, aimed at ensuring the improvement of the physical and chemical properties of the soil and raising its fertility, completing the requirement of assimilable nutrients according to the requirements of the species, rootstocks, cultivar/rootstock associations in relation to age and vegetation phases of plants (Asănică & Hoza, 2013). Among the main elements of the fertilization system in the modern tree nursery are: the accumulation of organic matter in the soil through crop rotations and the incorporation of special plant residues for green fertilizers; administration of mineral fertilizers with nitrogen, phosphorus, and potassium (Santos et al., 2023). The doses, terms and methods of fertilizer application are established differently for each sector of the nursery depending on the agrochemical properties of the soil and the requirements of the cultivated plants (Thomas, 2021).

The use of fertilizers in fruit trees culture becomes necessary to renew the reserve of nutrients consumed by plants or leached in depth, then to improve the physical condition, the chemical composition, and the general state of fertilization of the soil (Stănică, 2004).

In modern fruit growing, fertilization is one of the most important technological links (Braniște & Stănică, 2011). Due to their specificity, fruit trees plants occupy the same area of land for a long period of time, develop their root system to a considerable depth and due to the high productivity, they achieve, they extract from the soil, with the harvest, appreciable amounts of nutrients. Under these conditions, it is necessary to intervene every year, in several stages, with fertilizations that ensure, on the one hand, the achievement of a certain level of production, and on the other hand, a certain level and ratio of nutrients through returning the amounts of easily accessible nutrients extracted with the harvest to be able to maintain, in this way, the fertility of the soil in accordance with the age of the plantation and the level of production (Wallin, 2020).

The knowledge so far has proven that soil conditions, species, cultivar, rootstock, density, forecasted production etc. must be considered when applying fertilization. Establishing the optimal doses for each situation in the field must be done after analysing a series of soil properties, knowing the requirements imposed by the culture and those related to ensuring a certain quantitative and qualitative level of production.

To obtain high, constant, and quality productions in the nursery, a permanent control of the vegetation factors and especially of the nutritional ones is necessary. This control aims to maintain a balance between the main nutritional elements. The main objective of the research was to establish the influence of irrigation and fertilization on some physiological characteristics of plum in the nursery, in this case, the rate of photosynthesis. Through this research, the authors want to obtain a healthy, vigorous fruit tree planting material, superior to non-irrigated and non-fertilized nurseries.

MATERIALS AND METHODS

The research was carried out in the year 2022, in a private nursery. The nursery includes tree planting material of the following species: apple, pear, plum, apricot, cherry, peach, almond, cherry, quince, and walnut.

The study was carried out based on a trifactorial experiment of the 4 x 2 x 4 type, organized in five repetitions, with plots comprising four trees planted at 0.7 x 0.25 m, with irrigation as the primary factor, cultivar as a secondary factor and fertilization as tertiary factor. To obtain the doses of nitrogen; phosphorus: potassium (NPK) related to the fertilization treatments, complex fertilizer 16:16:16 was used, in the following amounts (kg/ha): 50 kg for $N_8P_8K_8$; 100 kg for $N_{16}P_{16}K_{16}$; 150 kg for $N_{24}P_{24}K_{24}$. Regarding irrigation, 4 watering norms were used, respectively non-irrigated, irrigated with 10 mm, irrigated with 20 mm and irrigated with 30 mm. The plum cultivars studied were ‘Stanley’ and ‘Cacanska Lepotica’. As general characteristics of the ‘Stanley’ cultivar, it can be mentioned the tolerance to viruses, it is partially self-fertile and a good pollinator for other cultivars like ‘Rivers’, ‘Agen’, ‘Anna Späth’. It is very productive and the production is constant. The trees are of medium vigour, with fruiting predominantly on May bunches. The fruit are medium, with a narrow and deep ventral furrow evident throughout the height of the fruit. ‘Stanley’ is heat demanding, it does well up to 500 m altitude, very precocious and productive. The tree of the ‘Cacanska Lepotica’ is of medium vigour, early, productive, partially self-fertile. The fruit is medium to large (45-50 g), ovoid, asymmetric, dark blue in colour. The flesh is yellowish-green, consistent, sweet-sour taste, non-adherent to the seed. Harvest maturity is the third decade of July. The rate of photosynthesis was calculated using CIRAS-3 equipment, which consists in closing a leaf in a closed, transparent flap and measuring the drop in CO_2 concentration. The pace of photosynthesis is not determined by a single formula, but rather by a complex process involving several reactions that occur in chloroplasts. The following formula can be used to summarize the enitre proces 6 H_20+ 6 CO_2 + light energy (captured by assimilatory pigments) = $C_6H_{12}O_6$ + 6 O_2. In the presence of sunshine, carbon dioxide and water are transformed into glucose and oxygen. Many variables, including light intensity and wavelenght, CO_2 concentration, temperature and nutrition availability affect the rate of photosynthesis. The obtained results of the measurements have been statistically processed. It was used the descriptive statistical analysis.

RESULTS AND DISCUSSIONS

Because the researches until now have been carried out in orchards, not in nurseries, the specialized literature is incomplete in this regard. The authors considered it useful to obtain information, through the present research, regarding the necessity and efficiency of applying localized irrigation to the culture of fruit trees in the nursery, against the background of different fertilization treatments. It can be observed that both cultivars and the irrigation or fertilization had a real and statistically ensured influence on the rate of photosynthesis in the seedlings from 2022 under the conditions of a homogeneity of the environmental conditions at the level of experience (Table 1).

Table 1. Analysis of variance regarding the effect of cultivar, irrigation, and fertilization on photosynthetic rate

Source of variation	SP SS	GL DF	S^2 MS	F test
Entire	127.53	159		
Repetitions	0.18	4	0.05	0.41
Irrigation	39.68	3	13.23	120.55**
Irrigation error	1.32	12	0.11	
Cultivar	1.44	1	1.44	8.80**
Irrigation x Cultivar	5.70	3	1.90	11.57**
Cultivar error	2.63	16	0.16	
Fertilization	43.58	3	14.53	203.46**
Irrigation x Fertilization	16.37	9	1.82	25.48**
Source of variation	SP SS	GL DF	S^2 MS	F test
Cultivar x Fertilization	2.68	3	0.89	12.53**
Irrigation x Cultivar x Fertilization	7.10	9	0.79	11.05**
Fertilization error	6.85	96	0.07	

Fertilization showed the highest contribution to the variability of the photosynthesis rate (47.26%), followed by irrigation (28%), both effects being significantly superior to the cultivar effect (2.04%) Also the single or double interactions between the factors showed significant influences on this character, with a higher contribution in the case of the combination of irrigation and fertilization, but considerably less than their separate effects.
The results obtained at the level of experience regarding the rate of photosynthesis of seedlings were influenced to a degree of approximately 8.6% by other uncontrollable sources of variation.
Regarding the unilateral effect of irrigation, the average rate of photosynthesis showed an amplitude of variation of 1.32 µmol $CO_2/m^2/s$, with average values between 2.08 in the case of the non-irrigated variant and 3.40 µmol $CO_2/m^2/s$ in the case of applying the watering norm of 30 mm, under the conditions of a high variability of 21.52% between the four irrigation treatments (Table 2).
At the level of the whole experience in this year's climatic conditions, irrigation showed a significant effect on photosynthesis related to increases between 14.75 and 63.61 %. Increasing the watering rate from 10 to 20 mm significantly influenced this character on the background of an intensification of photosynthesis by 19.3%, while changing the watering rate from 10 to 30 mm, caused a significant increase by 42.6% of this process.

Table 2. Average photosynthetic rate under the effect of the different watering norms

Watering norm	Photosynthetic rate (μmol $CO^2/m^2/s$)		Relative values (%)	Difference/ Significance
0-10 mm	2.08	2.38	114.75	0.31**
0 -20 mm	2.04	2.84	136.87	0.77***
0-30 mm	2.08	3.40	163.61	1.32***
10-20 mm	2.38	2.84	119.28	0.46***
10-30 mm	2.38	3.40	142.59	1.01***
20-30 mm	2.84	3.40	119.54	0.56***

DL $(LSD)_{5\%}$=0.16 DL $(LSD)_{1\%}$=0.23
DL $(LSD)_{0,1\%}$=0.32

Considering the cumulative effect of the cultivar this year average values of the photosynthesis rate were found with limits from 2.61 μmol $CO_2/m^2/s$ in the case of the 'Stanley' cultivar to 2.74 μmol $CO_2/m^2/s$ in the case of the 'Cacanska Lepotica'. As such, in general the seedlings of the 'Cacanska Lepotica' presented a more intense photosynthesis by approximately 5.1%, without the respective difference reaching the level of significance (Table 3).

Table 3. Average photosynthetic rate of the two cultivars

Cultivar	Photosynthetic rate (μmol $CO_2/m^2/s$)		Relative values (%)	Difference/ Significance
'Cacanska Lepotica' - 'Stanley'	2.74	2.61	105.14	0.13

DL $(LSD)_{5\%}$=0.14; DL $(LSD)_{1\%}$=0.19; DL $(LSD)_{0,1\%}$=0.26.

The average photosynthesis rate values of the seedling leaves under the effect of different doses of NPK showed an amplitude of 1.44 μmol $CO_2/m^2/s$, with limits from 1.99 in the case of unfertilized agricultural fund to 3.44 μmol $CO_2/m^2/s$ for the treatment with 24 kg of NPK, against the background of high variability between treatments (Table 4).

Table 4. Average photosynthetic rate under the effect of the different fertilization

NPK dose	Photosynthetic rate (μmol/m^2/s)		Relative values (%)	Difference/ Significance
$N_0P_0K_0$ - $N_8P_8K_8$	1.99	2.49	124.80	0.49***
$N_0P_0K_0$ -$N_{16}P_{16}K_{16}$	1.99	2.78	139.41	0.79***
$N_0P_0K_0$ -$N_{24}P_{24}K_{24}$	1.99	3.44	172.44	1.44***
$N_8P_8K_8$ -$N_{16}P_{16}K_{16}$	2.49	2.78	111.71	0.29***
$N_8P_8K_8$ -$N_{24}P_{24}K_{24}$	2.49	3.44	138.17	0.95***
$N_{16}P_{16}K_{16}$ -$N_{24}P_{24}K_{24}$	2.78	3.44	123.69	0.66***

DL $(LSD)_{5\%}$=0.12; DL $(LSD)_{1\%}$=0.16; DL $(LSD)_{0,1\%}$=0.20

Compared to the non-fertilized version, it is found that the application of different doses of NPK allowed a significant intensification of photosynthesis by 2.4-4.0%. The addition of fertilization from 8 to 16 kg favoured a significant increase of 11.7% in this process, while the change in the dose from 16 to 24 kg had a significant positive effect of 23.7%.
Regarding the interaction between cultivars and irrigation, in both cultivars, irrigation showed significantly positive influences on the rate of photosynthesis, against the background of higher effects on the 'Cacanska Lepotica' cultivar (Table 5).

Table 5. The effect of cultivar and irrigation on photosynthetic rate

Cultivar	Watering norm					
	0 mm	**10 mm**	**20 mm**	**30 mm**	$\overline{x} \pm s_{\overline{x}}$	$S_{\%}$
'Stanley'	z 2.07 a	y 2.44 a	y 2.55 b	x 3.36 a	2.61±0.08	28.56
'Cacanska Lepotica'	u 2.08 a	z 2.32 a	y 3.13 a	x 3.43 a	2.74±0.10	31.58
$\overline{x} \pm s_{\overline{x}}$	2.08±0.11	2.38±0.08	2.84±0.07	3.40±0.13	2.67±0.06	
$S_{\%}$	32.18	20.85	17.06	24.86	30.20	

Cultivar – DL $(LSD)_{5\%}$=0.24; DL $(LSD)_{1\%}$=0.32; DL $(LSD)_{0,1\%}$=0.43; (a,b)
Irrigation – DL $(LSD)_{5\%}$=0.27; DL $(LSD)_{1\%}$=0.37; DL $(LSD)_{0,1\%}$=0.51; (x,y,z,u)

Considering the effect of irrigation on the rate of photosynthesis in each cultivar it is observed that at 'Stanley' the values were between 2.07 μmol $CO_2/m^2/s$ for the non-irrigated version and 3.56 μmol $CO_2/m^2/s$ in the case of applying the norm of watering of 30 mm. Compared to the non-irrigated version, the application of the watering rules significantly influenced this process to a high extent of 17.86-62.02% (Table 6).

Table 6. The effect of irrigation on photosynthetic rate on the two cultivars

Watering norm	Photosynthetic rate (µmol $CO_2/m^2/s$)		Relative values (%)	Difference/ Significance
'Stanley'				
0-10 mm	2.07	2.44	117.86	0.37**
0-20 mm	2.07	2.55	123.07	0.48***
0-30 mm	2.07	3.36	162.02	1.29***
10-20 mm	2.44	2.55	104.42	0.11
10-30 mm	2.44	3.36	137.47	0.92***
20-30 mm	2.55	3.36	131.65	0.81***
'Cacanska Lepotica'				
0-10 mm	2.08	2.32	111.70	0.24*
0-20 mm	2.08	3.13	150.70	1.05***
0-30 mm	2.08	3.43	165.24	1.36***
10-20 mm	2.32	3.13	134.91	0.81***
10-30 mm	2.32	3.43	147.93	1.11***
20-30 mm	3,13	3,43	109.65	0.30*

DL $(LSD)_{5\%}$=0.24; DL $(LSD)_{1\%}$=0.32; DL $(LSD)_{0,1\%}$=0.43

Changing the watering rate from 10 to 20 mm was associated with a slight increase in the rate of photosynthesis by only 4.4%. Instead, the increase in the watering rate from 20 to 30 mm generated a significant increase of 31.65%.

In the case of seedlings of the 'Cacanska Lepotica', the variability between the effects of irrigation was associated with an amplitude of 1.36 µmol $CO_2/m^2/s$, ranging between 2.08 µmol $CO_2/m^2/s$ in the absence of watering and 3.43 µmol $CO_2/m^2/s$ for the norm of 30 mm. The three watering norms generated significant increases in photosynthesis rate associated with increases of 11.7-65.2%. The addition of irrigation from 10 to 20 mm determined a significant increase of 34.9% of this process, while the change of dose from 20 to 30 mm showed a significantly positive influence of 9.6%.

Considering the effect of the cultivar on the rate of photosynthesis on different watering rates, amplitudes are found from 0.01 µmol $CO_2/m^2/s$ for the non-irrigated variant to 0.58 µmol $CO_2/m^2/s$ for the variant related to the watering norm with 30 mm. Against the background of the application of the norm of 20 mm, the seedlings of the 'Cacanska Lepotica' showed a significantly higher intensity of this process by 22.75%, while the seedlings of the 'Stanley' showed a higher value by 5 % under the conditions of irrigation with 10 mm. Under the aspect of the intensity of photosynthesis, the seedlings of the two cultivars capitalized at a similar level the conditions of the non-irrigated agricultural fund and the one related to the 30 mm norm. Based on the exponential regression, it can be observed that in the case of the 'Cacanska Lepotica' the rate of photosynthesis showed an average growth rate of 0.045 µmol $CO_2/m^2/s$ for each mm of watering. The respective estimates have a precision of 95%, under conditions of increases of approximately 2.05 µmol $CO_2/m^2/s$ in the absence of irrigation (Figure 1).

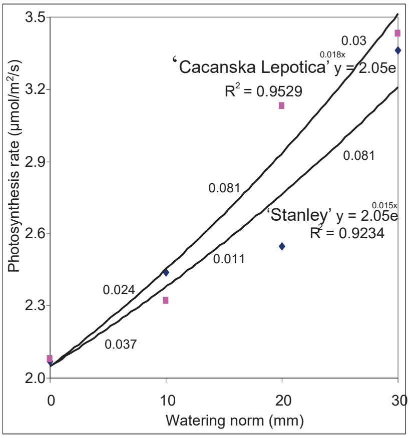

Figure 1. Variation of photosynthetic rate for the two cultivars under the effect of different watering norms

For the 'Stanley' cultivar, the effect of irrigation showed an average photosynthesis growth rate equivalent to 0.043 µmol $CO_2/m^2/s$ per mm, against the background of a lower variation (0.011) for the first two watering rates and a high variation (0.081) between the norms of 20 and 30 mm. The predictability of the logarithmic regression between the watering rate and the rate of photosynthesis in the Stanley cultivar is92.3%, based on a similar initial value in the absence of irrigation.

Considering the combined effect of irrigation and fertilization on the intensity of photosynthesis it is found that in the absence of watering, fertilization had the least influence on this process, while in the variant related to the watering norm of 30 mm, fertilization with different doses had a considerably higher influence (Table 7). In the conditions of the non-fertilized agricultural fund a variation of this character can be observed from 1.26 µmol $CO_2/m^2/s$ for the non-irrigated version to 2.62 CO_2µmol$/m^2/s$ for the watering rate of 30 mm.

Table 7. The effect of irrigation and fertilization on photosynthetic rate

Watering norm	NPK dose					
	$N_0P_0K_0$	$N_8P_8K_8$	$N_{16}P_{16}K_{16}$	$N_{24}P_{24}K_{24}$	$\overline{x} \pm s_{\overline{x}}$	$S_{\%}$
0 mm	u 1.26 d	z 1.91 c	y 2.17 d	x 2.97 c	2.08±0.11	32.18
10 mm	z 1.80 c	y 2.31 b	y 2.54 c	x 2.88 c	2.38±0.08	20.85
20 mm	z 2.30 b	y 2.94 a	y 2.89 b	x 3.23 b	2.84±0.07	17.06
30 mm	z 2.62 a	z 2.79 a	y 3.52 a	x 4.66 a	3.40±0.13	24.86
$\overline{x} \pm s_{\overline{x}}$	1.99±0.10	2.49±0.07	2.78±0.09	3.44±0.12	2.67±0.06	
$S_{\%}$	31.48	18.50	20.59	22.68	30.20	

Irrigation – DL $(LSD)_{5\%}$=0.25; DL $(LSD)_{1\%}$=0.34; DL $(LSD)_{0,1\%}$=0.44; (a,b,c)
Fertilization – DL $(LSD)_{5\%}$=0.24; DL $(LSD)_{1\%}$=0.31; DL $(LSD)_{0,1\%}$=0.41; (x,y,z,u)

On this farmland, irrigation generated significant increases in the photosynthesis rate of 43.43-108.37% compared to the non-irrigated version. Changing the watering norm by 10-20 mm determined a significant variation of 0.32-0.5 µmol $CO_2/m^2/s$ of this process (Table 8).

Under the effect of applying the treatment with 8 kg of NPK, the rate of photosynthesis was between 1.91 and 2.94 µmol $CO_2/m^2/s$. As such, in this case the watering norms had a significant effect, associated with increases of 0.4-1.03 µmol $CO_2/m^2/s$ on the intensity of this process. Only supplementing irrigation by changing the watering rate from 10 to 20 mm generated a significant 27.3% increase in photosynthesis. Against the background of fertilization with 16 kg of NPK, the application of irrigation showed considerable and significant effects of 17.1-62.6% related to variations of 0.37-1.36 µmol $CO_2/m^2/s$. Increasing the watering norms by 10 mm allowed a significant increase of this character by 14-21.8%.

Under the conditions of the agricultural fund fertilized with 24 kg of NPK, the same trends are manifested, but the irrigation effect is less, so that only the application of the 20-30 mm norms produced increases of 8.75-56.9% compared to the non-irrigated version. The addition of irrigation from 10 to 20-30 mm determined significant increases of 12.15-62.81 in the rate of photosynthesis.

Based on the exponential regression, it can be observed that in the case of the non-irrigated version, the rate of photosynthesis showed an average growth rate of 0.071 µmol $CO_2/m^2/s$ for each kg of NPK, with limits from 0.03 0.1. The respective estimates have a precision of 96.23 %, under the conditions of a value of 1.03 µmol $CO_2/m^2/s$ in the absence of fertilization (Figure 2).

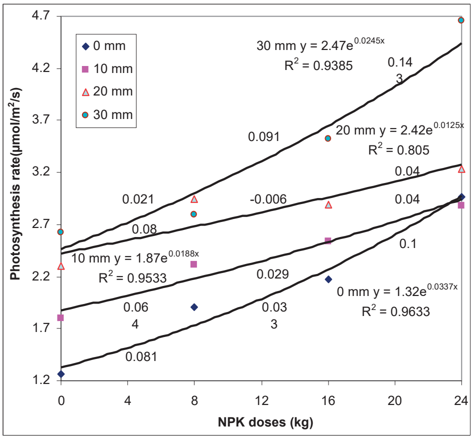

Figure 2. Variation of photosynthetic rate under the effect of different watering norms and fertilizations

For the watering norm of 10 mm, the effect of fertilization on the intensity of photosynthesis is expressed by a linear regression that is based on a coefficient of determination of 95.33% and indicates a smaller variation of this process between the doses of 8-16 kg NPK.

Under the effect of the watering norm of 20 mm, fertilization showed an influence of 80.5 % on the rate of photosynthesis, against the background of a value of 2.42 µmol $CO_2/m^2/s$ in the non-fertilized version and a small variation between doses of 8 and 16 kg NPK.

In the conditions of irrigation with 30 mm, there is an intensification of photosynthesis proportional to the dose of NPK, associated with an average rate of 0.085 µmol $CO_2/m^2/s$ per kg of NPK, in the conditions of a value of 2.47 µmol $CO_2/m^2/s$ in the absence of fertilization.

Regarding the effect of the interaction between cultivars and fertilization on the rate of photosynthesis, in the case of the 'Stanley' cultivar, the treatment with NPK was associated with a significant intensification of this process compared to the unfertilized variant and smaller variations between doses (Table 8).

Table 8. The effect of cultivar and fertilization on photosynthetic rate

Cultivar	NPK dose				$\bar{x} \pm s_{\bar{x}}$	S%
	$N_0P_0K_0$	$N_8P_8K_8$	$N_{16}P_{16}K_{16}$	$N_{24}P_{24}K_{24}$		
'Stanley'	z 2.01 a	y 2.52 a	y 2.59 b	x 3.31 b	2.61±0.08	28.56
'Cacanska Lepotica'	u 1.98 a	z 2.46 a	y 2.96 a	x 3.56 a	2.74±0.10	31.58
$\bar{x} \pm s_{\bar{x}}$	1.99±0.10	2.49±0.07	2.78±0.09	3.44±0.12	2.67±0.06	
S%	31.48	18.50	20.59	22.68	30.20	

Cultivar – DL $(LSD)_{5\%}$=0.19; DL $(LSD)_{1\%}$=0.26; DL $(LSD)_{0,1\%}$=0.33; (a,b)
Fertilization – DL $(LSD)_{5\%}$=0.17; DL $(LSD)_{1\%}$=0.22; DL $(LSD)_{0,1\%}$=0.29; (x,y,z)

Also, the 'Cacanska Lepotica' effectively capitalized on the NPK treatments that determined significant increases in terms of the intensity of photosynthesis compared to the unfertilized variant, against the background of significant differences between the treatments as well.

Considering the information, it is found that in the case of 'Stanley', the effect of fertilization on photosynthesis can be estimated by means of an exponential regression, with a precision of 92.98%. As such, the rate of photosynthesis increased proportionally with the dose of NPK with rates between 0.009-0.09 µmol $CO_2/m^2/s$ per kg NPK.

The relationship between the dose of NPK and the rate of photosynthesis in seedlings of the 'Cacanska Lepotica' is highlighted with a precision of approximately 99.8 % by means of an exponential function. Thus, against a value of 1.99 µmol $CO_2/m^2/s$ in the absence of fertilization, the average growth rate of this process was 0.066 µmol $CO_2/m^2/s$ per kg NPK applied, with different values from one dose to another of 0.063-0.075 µmol $CO_2/m^2/s$ per kg NPK. Following the comparison of the photosynthesis rate of the two cultivars on different fertilization treatments, it is found that on the unfertilized agro-fund and under the effect of the dose of 8 kg of NPK, the variation was very low and not statistically ensured. The seedlings of the 'Cacanska Lepotica' utilized the fertilization with 16 and 24 kg NPK at a significantly higher level, achieving a photosynthesis rate increases by 7.55-14.29 % (Table 9).

Table 9. The effect of cultivar on photosynthetic rate under different fertilizations

Cultivar x NPK	Photosynthetic rate (µmol $CO_2/m^2/s$		Relative values (%)	Difference/ Significance
$N_0P_0K_0$				
'Cacanska Lepotica' - 'Stanley'	1.98	2.01	98.51	-0.03
$N_8P_8K_8$				
'Cacanska Lepotica' - 'Stanley'	2.46	2.52	97.62	-0.06
$N_{16}P_{16}K_{16}$				
'Cacanska Lepotica' - 'Stanley'	2.96	2.59	114.29	0.37***
$N_{24}P_{24}K_{24}$				
'Cacanska Lepotica' - 'Stanley'	3.56	3.31	107.55	0.25*

DL $(LSD)_{5\%}$=0.19; DL $(LSD)_{1\%}$=0.26; DL $(LSD)_{0,1\%}$=0.33

Considering the interaction between fertilization and the rate of photosynthesis for the 'Stanley' cultivar, it is observed that NPK fertilization generated significant increases (25.37-64.68%) in the intensity of this process. Also, only the dose changes from 8 and 16 to 24 kg NPK was associated with a significant increase in the rate of photosynthesis by 27.80-31.35% (Table 10).

Under the effect of different fertilization treatments, seedlings of the 'Cacanska Lepotica' cultivar recorded a rate of photosynthesis with limits from 1.98 µmol $CO_2/m^2/s$ in the case of the unfertilized variant, up to 3.56 µmol $CO_2/m^2/s$ for the variant with 24 kg NPK, against the background of a variability between treatments of 31.58%.

Compared to the non-fertilized agricultural fund, NPK treatments had a significantly higher efficiency materialized by increases of 24.24-79.80%. Changing the dose of NPK from 8 to 16 and 24 kg, respectively, was associated with significant increases of 20.33-44.72% in the intensity of photosynthesis.

Table 10. The effect of fertilization on photosynthetic rate of the two cultivars

NPK dose x Cultivars	Photosynthetic rate (µmol $CO_2/m^2/s$)		Relative values (%)	Difference/Significance
'Stalney'				
$N_8P_8K_8$ – $N_0P_0K_0$	2.52	2.01	125.37	0.51***
$N_{16}P_{16}K_{16}$– $N_0P_0K_0$	2.59	2.01	128.86	0.58***
$N_{24}P_{24}K_{24}$ – $N_0P_0K_0$	3.31	2.01	164.68	1.30***
$N_{16}P_{16}K_{16}$ – $N_8P_8K_8$	2.59	2.52	102.78	0.07
$N_{24}P_{24}K_{24}$ – $N_8P_8K_8$	3.31	2.52	131.35	0.79***
$N_{24}P_{24}K_{24}$ – $N_{16}P_{16}K_{16}$	3.31	2.59	127.80	0.72***
'Cacanska Lepotica'				
$N_8P_8K_8$ – $N_0P_0K_0$	2.46	1.98	124.24	0.48***
$N_{16}P_{16}K_{16}$– $N_0P_0K_0$	2.96	1.98	149.49	0.98***
$N_{24}P_{24}K_{24}$ – $N_0P_0K_0$	3.56	1.98	179.80	1.58***
$N_{16}P_{16}K_{16}$ – $N_8P_8K_8$	2.96	2.46	120.33	0..50***
$N_{24}P_{24}K_{24}$ – $N_8P_8K_8$	3.56	2.46	144.72	1.10***
$N_{24}P_{24}K_{24}$ – $N_{16}P_{16}K_{16}$	3.56	2.96	120.27	0.60***

DL (LSD)$_{5\%}$=0.17; DL (LSD)$_{1\%}$=0.22; DL (LSD)$_{0,1\%}$=0.29

Regarding the combined effect of the three factors, in the absence of irrigation in the case of the 'Stanley' cultivar, fertilization showed a significant influence on the rate of photosynthesis associated with increases of 0.68-1.32 µmol $CO_2/m^2/$ s (Table 11).

Table 11. The effect of irrigation and fertilization on photosynthetic rate of the two cultivars

Specification	Watering norm			
	0 mm			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
'Stanley'	z 1.37 a	y 2.05 a	y 2.18 a	x 2.69 b
'Cacanska Lepotica'	u 1.14 a	z 1.77 a	y 2.15 a	x 3.25 a
	10 mm			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
'Stanley'	y 2.18 a	y 2.44 a	y 2.34 b	x 2.81 a
'Cacanska Lepotica'	z 1.42 b	y 2.18 a	x 2.73 a	x 2.95 a
	20 mm			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
'Stanley'	y 1.85 b	x 2.84 b	x 2.63 b	x 2.88 b
'Cacanska Lepotica'	z 2.75 a	y 3.04 a	y 3.15 a	x 3.58 a
	30 mm			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
'Stanley'	z 2.62 a	z 2.74 a	y 3.22 b	x 4.85 a
'Cacanska Lepotica'	z 2.61 a	z 2.83 a	y 3.82 a	x 4.47 b

Cultivar – DL (LSD)$_{5\%}$=0.39; DL (LSD)$_{1\%}$=0.52; DL (LSD)$_{0,1\%}$=0.67; (a, b)
Fertilization - DL$_{5\%}$=0.34; DL$_{1\%}$=0.44; DL$_{0,1\%}$=0.57; (x, y)
DL (LSD)$_{5\%}$=0.37; DL (LSD)$_{1\%}$=0.49; DL (LSD)$_{0,1\%}$=0.63

For cultivar 'Cacanska Lepotica, the effect of fertilization is more obvious, associated with significant variations (0.63-2.11 µmol $CO_2/m^2/s$) both compared to the unfertilized variant and between treatments. Seedlings of the 'Cacanska Lepotica' showed a significantly

higher intensity of photosynthesis compared to the 'Stanley' cultivar, under the effect of fertilization with 24 kg of NPK.
In the case of the agricultural fund where 10 mm irrigation was applied, only fertilization with 24 kg of NPK favoured a significant 28.9% increase in the photosynthesis of the 'Stanley' cultivar compared to the non-fertilized version. The rate of photosynthesis in seedlings of the 'Cacanska Lepotica' under these irrigation conditions was significantly influenced by the fertilization treatment against the background of variations of 53.52-107.8%. In the absence of fertilization, the rate of photosynthesis in the seedlings of the 'Stanley' was significantly higher, while under the effect of fertilization with 16 kg of NPK, the seedlings of the 'Cacanska Lepotica' showed significantly higher values. The two cultivars utilized fertilization with 8 and 24 kg of NPK to the same extent. On the agricultural fund irrigated with 20 mm, fertilization with 8-24 kg of NPK of the 'Stanley' determined a significant increase in the intensity of photosynthesis by 0.99-1.03 μmol $CO_2/m^2/s$, while in the seedlings of the 'Cacanska Lepotica' the fertilization had a lower influence associated with significant increases only in the case of doses of 16-24 kg. The seedlings of the 'Cacanska Lepotica' showed a significantly higher intensity of photosynthesis compared to the 'Stanley', both in the absence of irrigation and under the effect of NPK fertilization.
In the case of applying the 30 mm norm, the varieties capitalized to the same extent on the conditions of the unfertilized agro-fund and the treatment with 8 kg of NPK. Under the effect of fertilization with 16 kg of NPK, seedlings of the 'Cacanska Lepotica' recorded a significantly higher value, while on the agricultural fund fertilized with 24 kg of NPK, significantly higher values of the photosynthesis rate were observed in the seedlings of the 'Stanley' cultivar. It is also found that fertilization had similar effects on the photosynthesis rate of the two cultivars.

CONCLUSIONS

Fertilization showed the highest contribution to the variability of the photosynthesis rate (47.26%), followed by irrigation (28%), both effects being significantly superior to the effect of the cultivar (2.04%), considering that in general the fruit trees cultivar 'Cacanska Lepotica' showed a more intense photosynthesis by about 5.1%. The application of irrigation had a significant effect on photosynthesis related to increases between 14.75 and 63.61%. Increasing the watering rate from 10 to 20 mm significantly influenced this character on the background of an intensification of photosynthesis by 19.3%, while changing the watering rate from 10 to 30 mm, caused a significant increase by 42.6% of this process. Compared to the non-fertilized version, it was found that the application of different doses of NPK allowed a significant intensification of photosynthesis by 24.872-4%. Supplementing fertilization from 8 to 16 kg favoured a significant increase of 11.7 % in this process, while changing the dose from 16 to 24 kg had a significant positive effect of 23.7%. Based on the application of the norm of 20 mm, the seedlings of 'Cacanska Lepotica' showed a significantly higher intensity of photosynthesis by 22.75%, while the seedlings of 'Stanley' showed a higher value by 5% under the conditions of irrigation with 10 mm. The fruit trees of 'Cacanska Lepotica' utilized the fertilization with 16 and 24 kg of NPK at a significantly higher level, achieving increases in the photosynthesis rate of 7.55-14.29 %. In the case of 'Stanley', the treatment with NPK was associated with a significant intensification of this process compared to the unfertilized variant and smaller variations between doses.

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IS SYMPTOMATOLOGY A RELIABLE TOOL FOR PLUM POX VIRUS MONITORING?

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Abstract

Sharka disease, caused by Plum pox virus (PPV), represents an important economically issue of stone fruits growers in Romania. Establishing plum orchards with PPV-free planting material, followed by their virus monitoring and removing infected trees, can contribute to PPV containment. Although PPV monitoring based on symptoms developed combined with serological or molecular assays is recommended for accurate virus detection, such approach is not costly effective in orchards. Therefore, there is under question whether a well recognizing of PPV symptoms developed by infected plum trees can be an acceptable tool for virus monitoring in orchards. To get this information, twenty-seven plum orchards comprising a large assortment of cultivars were surveyed. A total of 540 samples were tested by DAS-ELISA and compared with results of visual observation. Overall results revealed a high coincidental data of PPV infection established by serological detection and virus-based symptoms, suggesting that a good knowledge of PPV symptoms developed by infected trees on leaves could be a reliable tool for virus monitoring large areas of plum orchards.

Key words: *DAS-ELISA, plum, PPV symptoms, Sharka disease, survey.*

INTRODUCTION

Symptoms represent the effect of viruses on growth and development of plants, and are defined as perceptible changes in the functions of plant host (Bos, 1970). In the present, viral pathogens can be identified very precisely by many high sensitivity techniques. However, in cases of some viruses, external symptoms represent the first sign of the presence of pathogen into a plant. Obviously, not all viruses express themselves through symptoms; some of them remain latent in plant for a while. When a virus causes significant changes in plants that affect growth or production it is considered economically important (Hull, 2004). This is the case of Sharka disease, caused by Plum pox virus (PPV), an important economically issue of stone fruits in many European countries (Barba et al., 2011; Cambra et al., 2006), including Romania (Minoiu, 1997; Zagrai et al., 2010). Sharka has been spreading for more than a century in different parts of the world, from Bulgaria (Atanasoff, 1932) to Mediterranean basin, Middle East, Western Europe, Asia, Africa (Egypt, Tunisia), North (Canada) and South (Chile, Argentina) America (Roy and Smith, 1994; Barba et al., 2011) becoming a global concern. PPV is one of the ten most widespread plant viruses in the world (Scholthof et al., 2011). Plum is one of the most stone fruit specie affected by Plum pox virus, the trees once infected often produce typical symptoms more or less visible on leaves and fruits. Usually, PPV express symptoms on leaves, fruits and seeds of susceptible stone fruits cultivars. The symptoms of PPV developed on leaves consist in chlorosis (pale rings, spots more or less diffuse), vein yellowing or leaf mottling, necrotic ring patterns, yellowish to olive green spots or bands (Levy et al., 2000; Llácer and Cambra, 2006; Zagrai and Zagrai, 2024). There are some plum cultivars infected by PPV that do not exhibit any symptoms on fruits, such as Blue free, Opal, Stanley (Hamdorf, 1986), Čačanská Rana (Zawadzka et al., 1998), Čačanská Najbolja, Opal, Hanita, Tuleu timpuriu (Paprstein et al., 2007). However, most of the plum cultivars susceptible to PPV are strongly affected by virus infection leading often to premature dropping of fruits, but also to fruit deformation, irregular rings or lines on skin and/or in pulp, scars, necrosis and gummosis, making the fruits unsuitable for consumption (Kamenova et al., 2010).

To get a profitable plum orchard, farmers must pay a special attention to PPV management. No doubt that the using PPV resistant cultivars is the most efficient strategy for its control (Ravelonandro et al., 2011; Scorza et al., 2013). Since the scarcity of resistant plum cultivars is still an obstacle in implementation of such strategy, the other prevention measures should not be neglected. Thus, PPV requires a special attention starting with the placement of the new orchards as far as possible from potential sources of virus infection. Then, the new established orchards by using PPV-free planting material, followed by their monitoring and removing infected trees in the first 5 years after planting is recommended (Zagrai et al., 2022). All these prevention methods can substantially contribute to reducing the PPV spreading, and consequently its economically impact.

Although PPV monitoring based on symptoms developed combined with serological or molecular diagnosis is known as a reliable tool, such strategy is not costly effective for virus monitoring in orchards and became not accessible for farmers. However, a good knowledge of PPV symptoms, developed especially on leaves, could allow any farmers to take action for limiting the spread and potential increasing damages in the own orchard. Therefore, there is under question whether a well recognizing of PPV typical symptoms developed by infected plum trees can be an acceptable tool for virus monitoring within the orchards.

MATERIALS AND METHODS

Field surveys

Since Sharka disease is considered the main limiting factor in the profitability of plum crops through the significant damages it causes in endemic areas, the field surveys were focused on typical PPV symptoms on leaves that allowed a preliminary assessment of PPV occurrence based on visual observations. Twenty-seven young plum orchards from ten counties of Romania comprising a large assortment of plum cultivars were surveyed during May-June of 2020 to assess the PPV viral status based on typical PPV symptoms on leaves in comparison with serological detection. Two blocks of one hundred trees each were delineated in diagonal within each orchard according to Figure 1, covering the entire range of cultivars.

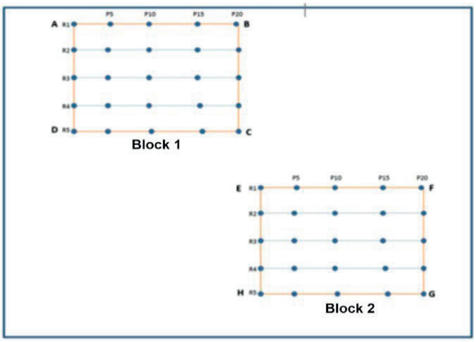

Figure 1. The design of blocks inside of plum orchards

A depth control was carried out within the blocks, each tree being checked for the presence of typical symptoms of PPV that could suggest a potential viral infection (Figure 2). Then, the incidence of PPV was determined based on visual observations of symptoms developed.

Figure 2. Example of typical PPV symptoms on plum leaves

Serological assay

Ten trees from each block were sampled for PPV detection using serological assays, as follows: if the visual incidence of PPV within the block was below 10%, one symptomatic sample and nine symptomless samples were taken; when the visual incidence of PPV was between 10% and 20%, two symptomatic and eight symptomless samples were taken; and so on, if the visual incidence was between 80%

and 90%, nine symptomatic samples and one symptomless sample were taken; and if the visual incidence was between 90% and 100%, only symptomatic samples were taken. Each sample consisted of a minimum ten leaves. The samples were transported under appropriate conditions to the laboratory, kept in the refrigerator and analysed within 1-5 days or stored at minus 24°C for maximum 30 days and subsequently subjected to laboratory testing.
Serological assays for PPV detection were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) using commercial polyclonal antiserum against PPV (Figure 3), according to the manufacturer's instructions (Bioreba, Switzerland).
Absorbance values were measured at 405 nm after 1h substrate hydrolysis. In the cases when absorbance values were more than twice of negative control, samples were considered positive. The samples were also tested for other viruses (data not shown).

Figure 3. Aspects of DAS-ELISA test at FRDS Bistrița

PPV incidence
PPV infections in the plum orchards were determined by correlating the preliminary assessment based of visual observations by the presence of typical PPV symptoms on leaves with the results obtained by serological diagnosis. When PPV infections based on visual monitoring were confirmed by serological diagnosis, PPV infection was established based on visual symptoms. When correlations between visual and serological data were only partially correlated an adjustment was made according to serological results.

RESULTS AND DISCUSSIONS

Field surveys
The orchard number, location, cultivars and the age of plum trees taken in this study are shown in the Table 1. The PPV incidence determined by visual observation revealed that only one out of twenty-seven plum orchards showed no PPV symptomatic tree (orchard no. 22). In the other orchards the PPV incidence based on symptoms developed varied between 0.5% (orchard no. 25) and 78% (orchard no. 20). Thereby, in nine orchards (no. 12, 14, 16, 18, 21, 24, 25, 26, 27) the PPV incidence determined by visual observations was between 0.5-10%, six orchards revealed an incidence of PPV between 11-20% (no. 1, 2, 3, 4, 10, 23), three orchards have recorded a rate of PPV between 21-30% (no. 7, 15, 17), one orchard had PPV incidence between 31-40% (no. 13), other one orchard had a PPV rate between 51-60% (no. 11), three orchards recorded an incidence of PPV between 61-70% (no. 5, 8, 9) and three orchards revealed an infection with PPV between 71-80% (no. 6, 19, 20).

Serological assay correlated with visual monitoring
All PPV symptomatic samples with one exception coming from orchard no. 20 confirmed the presence of PPV by DAS-ELISA. In this orchard, sixteen out of twenty collected samples developed symptoms suggesting a PPV infection, while the other four samples were symptomless. Thus, the incidence of PPV in the orchard no. 20, based on symptoms developed, was determined at 78% (Table 1). DAS-ELISA tests revealed that one symptomatic sample was not the result of PPV infection, but with other virus (data not shown). Consequently, the PPV infection rate in this case was recalculated at 73%. However, it should be noted that in the orchard no. 20 there is concordance of 94% between PPV incidence determined by visual observation of symptoms developed and serological results.
When symptomless trees were tested, only two cases of non-coincidental data were found. Precisely, one symptomless sample collected

from orchard no. 8 and the other one from orchard no. 23 were found infected with PPV, while the others confirmed the PPV-free status. Thus, the PPV incidence in the case of orchard no. 8 was determined at 68.5% when considering visual monitoring, while the adjusted results based on serological assays revealed a PPV incidence at 73%, so the concordance between symptomatology and serological results was calculated at 94%.

Table 1. The incidence of PPV in young plum orchards from Romania

No.	County	Location	Cultivar	Age of trees	PPV incidence (%) based on symptoms	PPV incidence (%) based on DAS-ELISA	Concordance of PPV incidence determined by symptomatology and DAS-ELISA (%)
1	Bihor	Buduslau	Topend plus, Jofela	3	16	16	100
2	Bistrita-Nasaud	Dumitra	Stanley, President, C. Lepotica	1	19	19	100
3	Bistrita-Nasaud	Jelna	Stanley, Topend plus	1	14	14	100
4	Bistrita-Nasaud	Ciceu-Mihaiesti	Stanley, Topend plus	4	16.5	16.5	100
5	Cluj	Cluj-Napoca	Topend	6	61.5	61.5	100
6	Cluj	Cluj-Napoca	Topend	6	77.5	77.5	100
7	Cluj	Tritenii de Sus	Stanley, D'Agen	4	24	24	100
8	Hunedoara	Turdas	Anna Spath, Stanley	3	**68.5**	**73**	**94**
9	Hunedoara	Brad	Tuleu gras, Stanley	3	61	61	100
10	Hunedoara	Ribita	Tuleu gras, Stanley, Anna Spath	1-3	15.5	15.5	100
11	Mures	Reghin	Haganta, President, Blue free	5	50	55	100
12	Mures	Reghin	Tophit, Cacak	5	5	5	100
13	Satu-Mare	Cehal	Stanley, C. Lepotica, Blue free, Centenar	1	39	39	100
14	Satu-Mare	Cehal	Stanley, C. Lepotica	3	9.5	9.5	100
15	Satu-Mare	Sacaseni	Stanley	2	24	24	100
16	Satu-Mare	Sacaseni	Stanley	2	1	1	100
17	Bacau	Itcani	Stanley, Anna Spath, D'Agen, Centenar	4	23.5	23.5	100
18	Bacau	Plopana	Stanley, President	1	0.5	0.5	100
19	Iasi	Scobinti	Stanley, D'Agen, Renclod Althan, Centenar	4	79	79	100
20	Iasi	Iasi-Bucium	Stanley, D'Agen, Centenar	8	**78**	**73**	**94**
21	Iasi	Podu Iloaiei	Tophit, Haganta, Hanita, Topend plus	2	2.5	2.5	100
22	Iasi	Popesti-Padureni	Victoria, Opal	4	0	0	100
23	Neamt	Icusesti	Stanley, Centenar, Dorin, Dambovita	3	**12.5**	**15.5**	**81**
24	Vaslui	Husi	Stanley, President, C. Lepotica	2	6	6	100
25	Vaslui	Husi	Blue free	2	0.5	0.5	100
26	Vaslui	Grumezoaia	Stanley, President, Grossa di Felisio	1	7	7	100
27	Vaslui	Crasna	Stanley, Anna Spath, D'Agen, Centenar	3	6	6	100

In a similar way, 81% concordance between PPV incidence determined by visual symptoms and serological results was calculated at orchard no. 23. It should be highlighted that a perfect coincidental PPV incidence determined by visual observation of typical PPV symptoms and virus detection by DAS-ELISA was established in most of the surveyed orchards (24 orchards out of 27). Moreover, the average rate of PPV infection in all 27 plum orchards (Figure 4) settled by visual inspections (27.6%) was similar with that determined considering DAS-ELISA test (27.7%).

Although there were sporadically cases when PPV infections were not found by both methods, it can be highlighted that the differences between PPV incidences assessed by visual observations and serological tests are insignificant. It should be mention that the field observations were made just one time during the vegetative period and hence a possible missing of symptoms developed by some infected cultivars in such period. Therefore, by increasing the number of surveys, it could reduce even such discrepancy.

Overall results revealed a high coincidental data of PPV infection established by visual monitoring of typical PPV symptoms developed on leaves and considering serological detection (Figure 5). This suggests that a good knowledge of PPV symptoms developed by infected plum trees on leaves could be used as a reliable tool for virus monitoring of large areas of plum orchards. Such monitoring can be very useful and accessible to any farmers interested to limit the PPV impact in their orchards by getting knowledge to virus symptoms expressed by plum cultivars. However, such approach is not at all recommended in activities related to plum propagation.

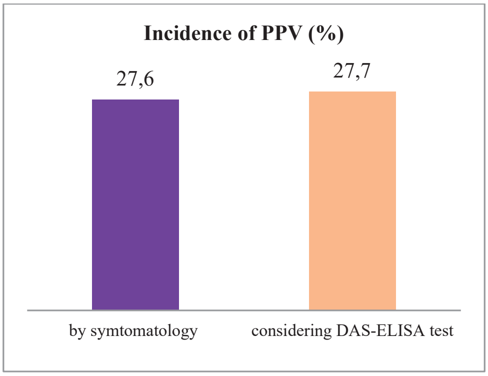

Figure 4. The average incidence of PPV (%) based on symptomatology versus taking into account serological results

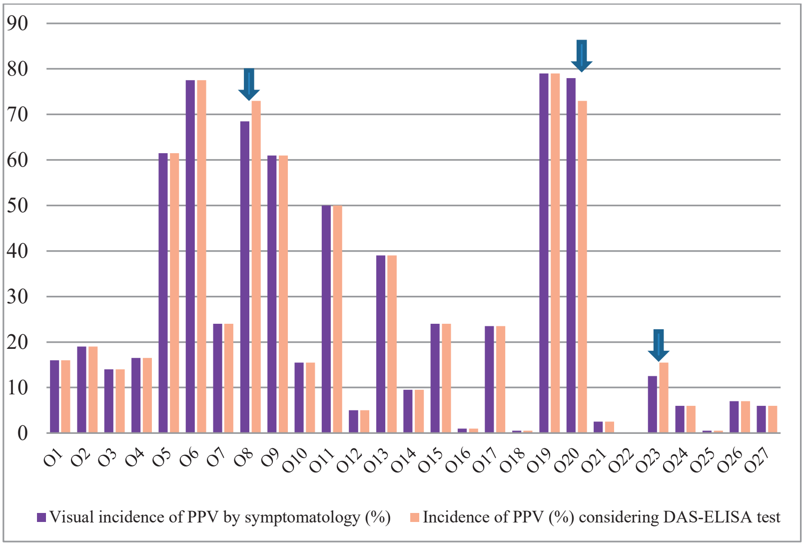

Figure 5. Comparison of PPV infection rate based on symptomatology versus serological results within each surveyed orchard

CONCLUSIONS

A high coincidental data of PPV infection was determined by serological detection and virus-based symptoms. Consequently, a well recognizing PPV symptoms developed by infected plum trees could be a reliable tool for PPV monitoring within the orchards, especially by farmers. If properly trained, they are the first that could identify potential issues of orchards, and to take proper measures in order to limit the spread of this economical important virus inside of orchard and out of it, to other proximal trees.

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IMPACT ON UV-VISIBLE SPECTROSCOPY PARAMETERS OF TAMAIOASA ROMANEASCA WINES FROM MUSTS CLARIFIED WITH PEA PROTEIN BASED FINING AGENTS

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Abstract

Pea protein is a plant-based fining agent recently approved for the clarification of musts and wines. Vegetal proteins are intended to replace the classical fining agents based on proteins of animal origin or the synthetic polymer polyvinylpolypyrrolidone (PVPP), which both proved very efficient for partial removal of polyphenols, but are less accepted by vegetarian or eco-friendly consumers. As an alternative, pea protein can be used for clarification either alone or in complex products containing other non-animal materials. This paper focuses on the evaluation of several pea protein based fining agents used to clarify the must of Tămâioasa românească, an aromatic grape variety which is vinified with a short maceration, leading to wines with a higher content of polyphenols. Variants with no fining as well as PVPP fining were also produced. For all fining variants, the clarification was performed both with oxygen protection and in the presence of oxygen. UV-visible spectroscopy was used to determine parameters related to the content of phenols in the resulted wines (total phenol index as OD 280 nm, flavonoids as OD 365 nm, CIELab parameters and colour differences), after must clarification and completion of the fermentation.

***Key words**: white wine; pea protein fining agents; CIELab, total phenol index*

INTRODUCTION

One new trend in food and beverages is a movement towards less processed products based on natural ingredients. Moreover, vegetarians and vegans demand for their wines that no animal product be used in winemaking (Goodman, 2023). Therefore, even fining agents, which technically are adjuvants and do not remain in the final wines (Kemp et al., 2022), have come into question, when they are of animal or synthetic origin. This is the case with PVPP (polyvinylpolypyrrolidone), a much appreciated synthetic polymer of pyrrolidone (Haaf et al., 1985), which is useful for removing fast some of the small polyphenol molecules, such as leucoanthocyanidins and catechins (Donner et al., 1993; Laborde et al., 2006), which are prone to oxidation and cause "browning" or "pinking" (Gil et al., 2017; Cojocaru and Antoce, 2019; Ugliano et al., 2021). PVPP is also known for reducing bitterness and astringency, through the same mechanism of polyphenol removal, even though it does not act on large polyphenol molecules, such as tannins. For these large polyphenol molecules, proteins are more effective fining agents (Cosme et al., 2008), especially the ones of animal origin, which were traditionally used and proved in time. Nowadays, there is interest in replacing PVPP and animal proteins with natural/vegan alternatives (Cosme et al., 2012; Versari et al., 2022), more acceptable from the viewpoint of many consumers. For this reason, several studies were recently done attempting to replace PVPP with vegetal proteins such as those from rice, potato, soy or pea (Gambuti et al., 2012; Kang et al., 2018; Marangon et al., 2019; Cojocaru and Antoce, 2022), as well as anorganic materials, such as activated carbon or bentonite. Some of these materials, used in single treatments, can lead to a reduction of other desirable compounds in beverages (Seriš et al., 2024).

There are variations in the polyphenol removing efficiency of these materials (Río Segade et al., 2020), as well as effects on aroma (Lambri et al., 2010; Vincenzi, et al., 2015) and colour (González-Neves et al., 2014), therefore tests are necessary to determine the optimum agents or combination of agents for each type and style of wine.

Regarding the allergenic potential, proteins of either animal or vegetal origin may be a concern, but in wine fining it was proven that usually the final wines do not contain allergenic proteins (Peñas et al., 2015). Moreover, plant proteins have a much lower allergenic potential and for this reason pea and potato protein fining agents need not be mentioned on the labels in accordance to the legislation of EU (Peñas et al., 2015; EGTOP, 2015).

In this study the d/or inorganic materials (activated carbon, bentonite).

The tests were performed on the must of the aromatic white variety Tamâioasa româneasca, which is alternatives tested were based on pea protein, combined also with two other materials. The two extra materials could be of non-animal organic materials (yeast hulls, chitosan) an known for having higher loads of polyphenols transferred from the seeds and skins, due to the maceration process carried out for aroma extraction (Stoica and Gheorghita, 2008).

MATERIALS AND METHODS

The grapes of Tamâioasă românească variety were harvested on September 11th, 2023 from Pietroasa wine region. At harvest the grapes had the parameters specific for the production of a quality wine: sugars 22.61 ºBx; glucose+fructose 213.1 g/l; density 1.0941 g/ml; pH=3.97; total acidity 5.52 g/l; volatile acidity 0 g/l; malic acid 2.33 g/l; lactic acid 0 g/l; tartaric acid 2.82 g/l; extract 33.7 g/l; assimilable 233 mg/l, ethanol 0.15%; polyphenols 862 mg/l, potassium 2617 mg/l.

The grapes were processed in the same day, starting with the destemming and crushing. The free-run must, which resulted from the grapes pressed in a pneumatic horizontal wine press after a short enzyme maceration of 6 h, was treated for the partial removal of the polyphenols with various fining agents. Volumes of 40 l of must were used for each variant and repetition. For the reductive process, the treatment, the racking from the deposit and the subsequent winemaking was performed in stainless steel tanks of 50 l, while glass demijohns were used for the oxidative process.

For the reductive process 3 repetitions were prepared for each variant. For the sake of comparison with a less controlled process, in which the presence of more oxygen is unavoidable, for each variant a single repetition in demijohns was prepared, too. The treatments were similar for the must in both type of recipients, as described in Table 1.

Table 1. Variants of must treatments and the fining agents used alone or in combinations

Variant name	PVPP	Pea protein (P)	Chito-san (K)	Yeast hulls (Y)	Car-bon (C)	Bento-nite (B)
V0	-	-	-	-	-	-
PV	☑	-	-	-	-	-
PP	-	☑	-	-	-	-
PYB	-	☑	-	☑	-	☑
PCB	-	☑	-	-	☑	☑
PCY	-	☑		☑	☑	-
PKC	-	☑	☑	-	☑	-
PKY	-	☑	☑	☑	-	-

Each treatment consisted of a dose of 20 g/hl of a fining agent or a combination of them. The fining agents used in this study are commercially available. PVVP SMARTVIN, chitosan Kitosmart and active carbon Acticarbone 2SW are from Enologica Vason (Italy), pea protein Proveget 100 is purchased from Agrovin (Spain), yeast hulls OENOLEES and calcium bentonite Microcol CL G are from Laffort (France).

After keeping the samples 24 h in the presence of the fining agents, each recipient was racked, separating the lees from the limpid must. Each variant and repetition were then inoculated with 25 g/hl *Saccharomyces cerevisiae* yeast and let for 10-14 days to complete fermentation to dryness. Fermentation activators were added in the beginning of the fermentation and again after 3 days of fermentation. In the stainless steel tanks the temperature was controlled and kept at around 15ºC during the entire period of fermentation. In the demijohns, even kept in a cool room, the temperature fluctuated between 15 and 20ºC. After the fermentation ceased, the wines were racked and sulfited with 50 mg/l sulfur dioxide. After one week the wines were racked again and analyzed.

Total phenolic index (TPI) was determined by measuring the optical density (absorbance) at

280 nm, the wavelength which is absorbed by the phenolic rings, especially from flavonoids such as catechins and condensed and hydrolysable tannins (Harbertson and Spayd, 2006). Flavonols, such as quercetin and kaempferol, absorb and can be estimated by measuring the optical density at 365 nm (Harbertson and Spayd, 2006).

The colour CIELab parameters were determined in accordance to the OIV method (OIV, 2021).

For absorbance and color measurements a UV-visible spectrophotometer Specord 250 (Analytik Jena, Germany) was used. Quartz cuvettes were used for the UV determinations and glass cuvettes for the visible spectrum determinations. For absorbance determinations a dilution of 10 was applied, therefore the final result was multiplied by the dilution factor. Whenever a cuvette with a smaller path length was used, the final result was also multiplied to adjust the final result for a cuvette of a 1 cm path length. For CIELab data acquisition and analysis the software WinAspect version 2.2.7 (Analytik Jena, Germany) was used.

CIELab parameters and their significance are described in detail in a previous paper (Antoce et al., 2022). Total colour difference (ΔE) was determined in accordance to the formula: $\Delta E = ((L_c-L_s)^2 + (a_c-a_s)^2 + (b_c-b_s)^2)^{1/2}$, where c=control and s=sample.

Statistical analysis was performed with the software package Origin 2018 (OriginLab, USA), applying, where appropriate, the statistical methods of Principal Component Analysis (PCA), one-way ANOVA, two-way ANOVA and post-hoc Tukey test.

RESULTS AND DISCUSSIONS

To estimate the impact of treatments on the overall polyphenol load, first the total phenolic index (TPI) was determined. For differences between samples within the same conditions (TPI_{red} and TPI_{ox}, respectively), one-way ANOVA was used for the calculations and Tukey test was applied for comparison of means. The differences induced by treatments and conditions, respectively, were calculated using two-way ANOVA and mean comparison by Tukey test at p<0.05. This analysis has confirmed that there is a significant difference between the total phenolic index of wines obtained in reductive and oxidative conditions, respectively. The differences between treatments, irrespective of the winemaking conditions, are shown in Table 2.

Table 2. Total phenolic index of wines made from must samples fined in reductive and oxidative conditions

Variant	TPI_{red}	TPI_{ox}	TPI differences induced by treatments, irrespective of winemaking conditions
	One-way ANOVA		Two-way ANOVA
V0	10.94±0.0003[a]	12.95±0.016[a]	a
PV	9.85±0.003[b]	12.26±0.011[b]	b
PP	10.31±0.006[c]	13.50±0.014[a]	c
PYB	10.65±0.004[d]	12.73±0.018[a]	c
PCB	10.35±0.002[c]	12.24±0.013[b]	bc
PYC	10.36±0.005[c]	12.85±0.020[a]	ac
PKY	10.57±0.002[d]	11.36±0.009[b]	bc
PKC	10.69±0.002[d]	11.80±0.083[b]	bc

Average values ± standard deviations and Tukey test for mean comparison (p<0.05); different letters represent significant differences between variants.

As it can be observed, all the applied treatments, irrespective of the winemaking conditions, have the ability to partly reduce the polyphenols contained in the Tămâioasa românească must, leading to wines with lower TPI compared to the control V0, which was not treated with any fining agent.

However, differences are also observable among the variants with specific fining agents or combinations. The treatments with PVPP, irrespective of the winemaking conditions, proved to be the most effective, significantly reducing the TPI, compared to all the other variants applied. In the used total dose of 20 g/hl, pea protein and its combinations with other fining agents reduced the TPI compared to control, but not as much as the PVPP in the same concentration.

As expected, the most obvious difference in the final wine TPI was induced by the winemaking conditions, the presence of oxygen leading to higher TPIs in all similarly-treated variants (Figure 1). It also proves that the oxidative conditions were not enough to lead to the precipitation of the oxidized polyphenols, which remained in the final wine, affecting the overall quality.

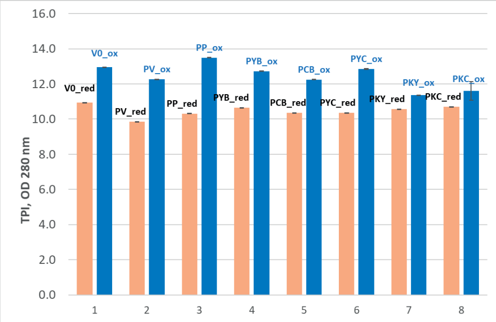

Figure 1. Total phenolic index (TPI) for wines resulted from must treated with various fining agents and combinations, in reductive (orange bars) and oxidative (blue bars) conditions

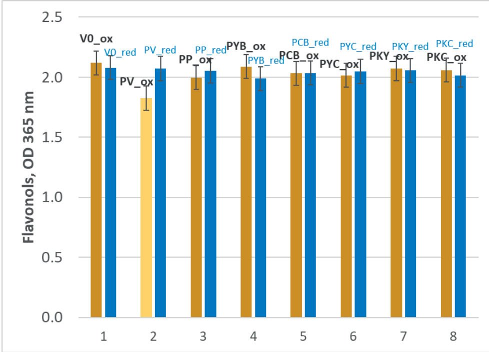

Figure 2. Flavonols content estimated as absorbance at 365 nm for the wine samples resulted from must treated with various fining agents

For the estimation of the flavonols in the final wines, it was similarly observed that the PVPP is the most effective in reducing the concentration of these compounds, compared with the same dose of pea protein and its combinations with other fining agents (Figure 2).

For an overall idea of how the treatments and winemaking techniques influence the wine colour, all the CIELab parameters were determined and are presented in Table 3.

Table 3. Colour CIELab parameters of wine must samples obtained from musts fined in reductive and oxidative conditions and differences calculated based on CIELab parameters *L*, *a* and *b*

Variant	L_{red}	L_{ox}	a_{red}	a_{ox}	b_{red}	b_{ox}	c_{red}	c_{ox}	h_{red}	h_{ox}	ΔE_{red}	ΔE_{ox}
V0	96.6±0.0	93.1	0.2±0.0	-0.3	8.9±0.0	15.3	8.9±0.0	15.3	1.5±0.0	-1.6		
PV	95.8±1.1	87.1	0.1±0.4	4.2	8.6±0.5	21.8	8.6±0.5	22.2	0.0±2.2	1.4	1.80±0.02	9.95
PP	95.7±1.2	87.5	0.3±0.1	5.0	8.3±0.2	22.6	8.3±0.2	23.1	1.5±0.0	1.4	1.81±0.63	10.63
PYB	96.4±1.3	90.3	0.1±0.1	3.2	8.7±0.1	20.7	8.7±0.1	20.9	0.5±1.8	1.4	1.81±0.52	7.04
PCB	95.8±1.4	93.7	0.1±0.1	2.0	8.4±0.7	13.6	8.4±0.7	13.7	0.5±1.8	1.4	1.27±0.05	2.88
PYC	97.4±0.0	85.8	0.2±0.0	4.7	7.7±0.0	21.4	7.7±0.0	21.9	1.5±0.0	1.4	0.73±0.02	10.80
PKY	94.5±0.0	93.0	0.6±0.0	0.9	9.3±0.0	17.2	9.3±0.0	17.2	1.5±0.0	1.5	0.73±0.04	2.29
PKC	96.6±0.0	89.6	0.2±0.0	3.6	8.5±0.0	21.5	8.5±0.0	21.8	1.5±0.0	1.4	1.17±0.02	8.11

Average values ± standard deviations

Luminosity L was not significantly affected by the treatment under reductive conditions, while some differences appeared in oxidative conditions, but mainly due to the delay in sedimentation of the particles in suspension.

Based on the CIELab parameters, the main difference between the oxidative and reductive winemaking is determined by the luminosity L, as it is shown by a Principal Component Analysis in which all reductive samples are grouped towards a lower impact of luminosity, because the samples are better clarified and L values are higher (Figure 3).

The extracted eigenvectors (Table 4) show that all the CIELab parameters are included in the component PC1, which accounts for 85.59% of the variance, but the Luminosity has an opposite impact (negative values) as compared to the parameters which relate to the actual colour (positive values).

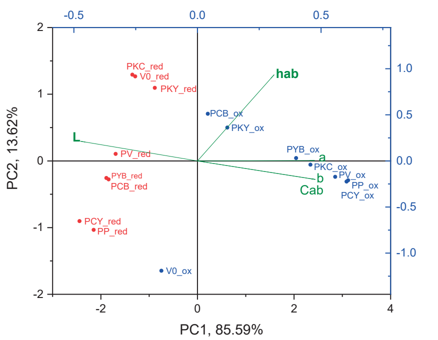

Figure 3. Principal Component Analysis plot based on CIELab parameters for wines resulted from must treated with various fining agents and combinations in reductive (red) and oxidative (blue) conditions.

Table 4. The extracted eigenvectors for the CIELab parameters

	Coefficients of PC1	Coefficients of PC2
L	-0.47379	0.21372
a	0.47418	0.00502
b	0.47604	-0.20116
C_{ab}	0.47722	-0.20007
h_{ab}	0.31036	0.93478

The other Principal Component, PC2, representing 13.62% of the total variance, is related mostly to the hue of the samples (h_{ab}). This means that the samples $V0_{red}$, PKC_{red}, PKY_{red} and PCB_{ox} and PKY_{ox} are associated with the deepest hues. This shows also the impact of the chitosan on the colour of the final wines, which retain generally more colour than the rest of the wines for which other fining treatments were used.

The effect on the colour is also more important when the oxidative winemaking is performed. This is easily observed in the colour space formed by the parameters *a* (variation of color from green to red) and *b* (variation between blue and yellow), where the samples produced in reductive conditions are clearly separated by the rest of the samples, having the lowest yellow and red components (Figure 4). The positive values of parameter *a* indicate that the colour is a shade of yellow and the positive values of parameter *a* indicate that the colour contains some red shades, but considering the very small values, these should be interpreted as giving the wine a brownish shadow, not red.

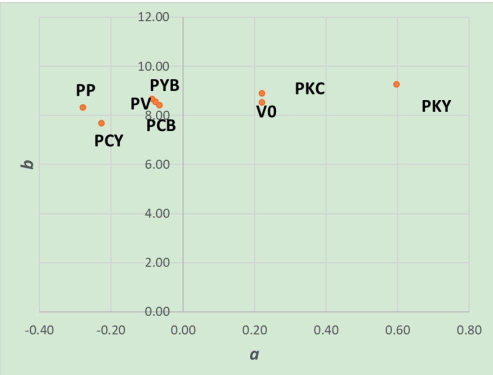

Figure 4. Placement in the colour space *a* (green to red) vs *b* (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in reductive (orange dots) and oxidative (blue dots) conditions

The effect of the treatments on the colour of the samples produced in a reductive way is low, the group of samples in Figure 4 being rather compact. However, the oxidative winemaking led to higher yellow and red components, with some differences among samples. The control oxidative sample was in this case the closest to the reductive samples group, showing that the fining treatments are not helping with regard to the quality of the final wines when winemaking is made in the presence of oxygen. Samples treated with pea protein in combination with chitosan and yeast (PKY) were also of a better colour in case of the oxidative conditions, as well as the samples treated with pea protein in combination with activated carbon and bentonite (PCB), which probably partially absorbed some of the oxidized components.

In order to differentiate the influence of the treatment type on the colour, in Figures 5 and 6 the samples vinified in reductive and oxidative conditions, respectively, are plotted in the space *a* vs. *b*.

For the samples prepared in reductive conditions (Figure 5) it can be observed that the most coloured, with a higher shade of red (giving overall a brownish shade) are the control wines, with no treatment and the wines obtained from musts treated with combinations of pea protein and chitosan (PKC and PKY). Taking into account the fact that the rest of combinations based on pea protein did not have positive values for the red shade, we can say that the chitosan is the fining agent responsible for adding this slight shift in the colour.

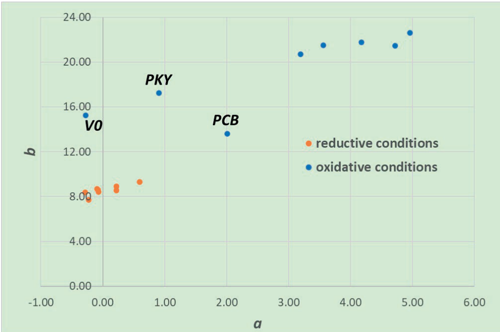

Figure 5. Placement in the colour space *a* (green to red) vs. *b* (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in reductive conditions

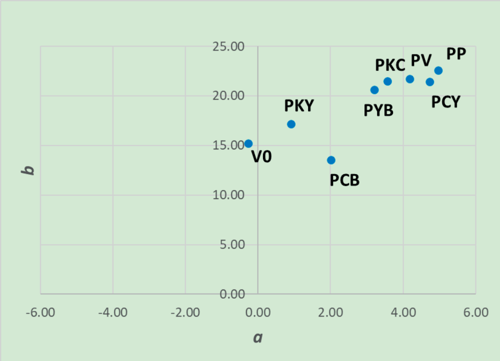

Figure 6. Placement in the colour space *a* (green to red) vs. *b* (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in oxidative conditions

For the samples prepared in oxidative conditions (Figure 6) the red-brownish shadow is present in all samples (small positive values for parameter *a* in all of them), irrespective of the type of fining agent. Thus, it can be said that, under these conditions, the treatment cannot influence too much the colour.

As for the chromaticity, it increases by a factor of 2-3 for the samples obtained in demijohns (*c* parameter being between 13.7-23.1) as compared to the ones produced in stainless steel tanks (*c* parameter between 7.7 and 9.3).

The overall colour difference measured for each sample against the control showed small differences for samples prepared in reductive conditions, but still noticeable for various treatments. Thus, a visible difference in colour, compared to control, is present for the samples treated with PVPP (PV), pea protein (PP) and the combination pea protein, yeast and bentonite (PYB), all having the calculated ΔE around 1.8, the differences being hardly perceivable for the rest of the samples (ΔE between 0.73-1.27). For the oxidative winemaking the differences in colour were very visible, some of them with ΔE over the value of 5, being clearly of another colour, here signifying uncontrolled oxidation. The only samples produced in demijohns which maintained a non-oxidized colour were those with values of ΔE up to 3, which is the case for the fining combinations PCB and PKY. These are the same samples with the ones underlined in Figure 3, due to the difference of red shade incorporated in parameter *a*.

CONCLUSIONS

Fining treatments based on pea protein achieve a partial removal of phenolic compounds and can be a replacement for the use of synthetic PVPP. To ensure quality of the final wine, the treatments and winemaking process need to be controlled and under reductive conditions.

In the presence of oxygen, the oxidized polyphenols are not sufficiently removed by any of the fining agents. These oxidized polyphenols also significantly change the colour of the final wine. Thus, in oxidative conditions, irrespective of the fining treatment, the values for TPI are higher, along with the values for *a* and *b* parameters of CIELab.

In reductive conditions and for the doses of the fining agents of 20 g/hl, the higher reduction of TPI and flavonoids is still achieved by the treatment with PVVP, which is more effective than all the other treatments. Good TPI and flavonoids reductions are possible by using pea protein (PP) or combinations of pea protein with activated carbon (PCB and PYC). Therefore, in case the wine is addressed to consumers who avoid synthetic products or are environmentally minded citizens, pea protein and its combinations with activated carbon represent suitable alternatives. Further tests are, however, required to determine the sensory impact and the effect of all these treatments on the aromatic compounds.

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PHENOLOGICAL AND SOME ENO-CARPOLOGICAL TRAITS OF THIRTEEN NEW ROMANIAN GRAPEVINE VARIETIES FOR WHITE WINE (*VITIS VINIFERA* L.) IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

Thirteen new grapevine varieties for white wines' behaviour was studied between 2015-2019, under climate change conditions in the ampelografic collection of the UASVM Bucharest. During the experimentation period, as compared to the reference period (1981-2010), the average temperature during the growing season increased by 0.75°C, the average annual maximum temperatures by 1.26°C, number of hot days by 21, number of very hot days by 6.2 and Huglin index by 140 units. Phenological cycles (budburst to harvest) varied between 138.6 and 140.6 days, with Aromat de Iaşi and Crâmpoşie selecţionată - the most precocious varieties and Columna - the latest variety, as an average of 5 years of experimentation. Due to the high temperatures during the growing season, phenological stages were anticipated, the harvesting being anticipated by approximately 15-25 days. The highest value of sugar content of must was for Aromat de Iaşi variety (23.33°Brix) and the lowest value for Astra (19.38°Brix). Aromat de Iaşi, Crâmpoşie selecţionată and Şarba varieties have been distinguished by the best qualities.

Key words: *climate change, grapevine, new cultivars, phenology.*

INTRODUCTION

The vine and wine sector is one of the most developed sectors in Romanian agriculture, which largely benefited from the country accession to European Union in 2007 (Antoce & Călugăru, 2017). Romanian viticulture is recognized by the diversity of varieties used for obtaining high quality wine products: white, red, aromatic wines etc. Along with the recovery of the ancient, autochthonous varieties, many new ones have been obtained, widening, this way, the assortment of different vineyards. Grapevine varieties' diversity is permanently completed with new varieties in order to satisfy consumer preference and adaptation to climate change, to winter frost and to globalization. In the last 5 decades, 87 new grapevine varieties were obtained in Romania, out of which 17 for white wines (Glăman et al., 2018).

In the breeding programs, there were used, as genitors of valuable autochthonous, varieties such as Fetească albă, Fetească regală, Grasă de Cotnari, Tămâioasă românească, Crâmpoşie etc, alongside foreign ones, such as Chardonnay, Sauvignon, Pinot gris, Riesling italian, Muscat Ottonel etc. Germplasm resources and genetic diversity in grapes is an important basis for new varieties development (Antoce et al., 2015).

The new created grapevine varieties belongs to different maturation groups and have generally intermediate behavior in relation to their genitors. If their obtaining aimed initially at widening options in wine production, and creation of new varieties, with earlier or later ripening, or more resistant to diseases, today the interest focuses on the behavior of these new varieties in the current climate context. In Romania the climate change manifests at the entire country level (Bucur & Dejeu, 2016), and causes consequences as well as at the global viticulture (Jones et al., 2005).

Many recent studies have been carried out on the effects of climate change on vines phenology (Bucur & Dejeu, 2018), grape production, its quality (Bucur & Dejeu, 2013) and climate suitability of Romanian wine growing regions (Irimia et al., 2017).

Global warming requires detailed studies of varieties' behavior under the new conditions, to adopt appropriate measures in order to mitigate its effects and to continue ensuring wines typicity.

Phenology is the first component affected by climate change and it is a key parameter for varietal adaption (Fraga et al., 2015; Garcia de Cortazar-Atauri et al., 2017).

Collecting data about grapevine phenological stages can help grapegrowers to take better decision in the vineyard to improve efficiency, reduced labor cost and to protect vine health. Thus, canopy management (pruning, shoots and cluster thinning, shoots trimming, leaf removal, phytosanitary treatments, irrigation, fertilization) is applied at very precise moments of it is nesessary to follow and anticipate phenological development. Also, when establish the news plantations, it is necessary to choose the varieties with certain stages of budbreak and ripening of grapes, according to the local climatic and soil conditions, in order to obtain quality yield.

Knowing the evolution of grape ripening (veraison-ripening interval), is very important for determining the time of grape harvest and for the quality of the wines according to the type of wine that is to be obtained. For a planning of this cultural practices, the growers need to know an advance the date of occurrence of the main phenological stades: budburst, flowering, veraison, grape ripening (Chuine et al., 2013; Verdugo-Vásquez et al., 2017).

The increase in air temperature leads to a faster deployment of the main phenophases (budbreak, flowering, veraison and ripening), changes in the physiological and biochemical processes of the plant, vegetative growth, production and quality. Many studies have reported the accelerating effects of rising temperature on grapevine phenology (Martínez-Lüscher et al., 2016).

In climate change conditions, temperature is the main factor that determines the anticipation of the phenological stages (Sadras & Pertie, 2011; Bellia et al., 2007). Along with temperature, the phenology of grapevine is also influenced by solar radiation, UV-B radiation, water availability, CO_2 concentration, geografic location, altitude, nitrogen status (Jones & Davis, 2000; Schultz, 2008; Martínez-Lüscher et al., 2016; Cola et al., 2017; De Rességuier et al., 2018; Alikadic et al., 2019).

Warmer conditions due to climate change are generally associated with shorter petriods between phenological events and to earlier harvest dates (Tomasi et al., 2011). Premature ripening happens under warm temperatures and interferes with the balanced accumulation of sugars, acids, aroma profiles and berry coloration (Zyprian et al., 2018).

The advance of phenological cycle as a result of climate change, with ripening period occurring under warmer climatic conditions can modify the characteristics of the berries, wich contain less anthocyanin, less acids, more sugars and less aroma compounds (Duchêne et al., 2014). The shift of phenology and advancement of maturity was also reported by Ranca et al., 2008; Rotaru et al., 2013; Stroe et al., 2013; Irimia et al., 2017; Bucur & Dejeu, 2018.

Numerous comparisons between varieties, in terms of phenological development, in different wine regions of the world are analyzed in the scientific literature (Bellia et al., 2007; Rustioni et al., 2014a; Orlandi et al., 2015; Zapata et al. 2016; Verdugo-Vásquez et al., 2017).

Sadras et al., 2009, reported a high plasticity of budburst and flowering associated with high yield plasticity.

The knowleges about the phenotipic diversity of grapevine varieties or to be obtained by crossing opens new perspectives to mitigate the effect of climate warming on grapevine behaviour and grapes composition (Zyprian et al., 2018; Bigard et al., 2018). Torregrosa et al. (2017) proposed a program for the selection of varieties that limit the accumulation of sugars in berries, while maintaining other qualitative compounds.

In this study, there were followed the phenology and performance of 13 new white grape varieties created in Romania, according to the protocol established under COST Action FA 1003 (Rustioni et al., 2014b). The current values, averages for the 2015-2019 time period, are compared with averages for the 1981-2010 in order to find consequences determined by climate change in their behavior.

MATERIALS AND METHODS

Plant material. The current study was carried out at the grapevine collection located in the Southern part of Romania, at the University of Agronomical Science and Veterinary Medicine Bucharest (N Lat.: 44° 47' 07"; E Long.: 26° 07' 28"; alt. 87 m). The vines were planted at a distance of 2.2 x 1.2 m (3787 vines/ha), grafted on the Kober 5 BB rootstock, with spur pruned cordon on demi-high stem (0.6-0.7 m).

The new varieties analyzed in this study, all of them for white wine production (Table 1) are: green-yellow coloured varieties (Alb aromat, Aromat de Iași, Astra, Blasius, Columna, Crâmpoşie selecționată, Donaris, Furmint de Miniş, Miorița, Șarba), rose coloured varieties (Roz de Miniș, Selena) and grey coloured variety (Băbească gri).

The methodology for phenotyping, sampling, measurements and the methods for sugar content (by refractometer in °Brix values) and juice acidity (g tartaric acid. L^{-1}) followed the standardized protocols for phenotyping berry enological traits ratified by COST Action FA1003 project "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" (Rustioni et al., 2014).

The sugar/acidity balance was estimated on the basis of the Brix/acidity ratio, that was obtained by dividing the °Brix value by % titratable acidity expressed as tartaric acid. All the activities were performed in four consecutive years, from 2015 to 2019.

Table 1. Grape varieties for white wine studied (Bucharest, 2015-2019)

Crt. no.	Cultivar	VIVC* no	Genitors	Color of berry skin
1	Alb aromat	23101	Tămâioasă românescă x IP165	green yelow
2	Aromat de Iași	20876	Fetească regală x Pinot gris	green yelow
3	Astra	632	Tămâioasă românescă open pollination	green yelow
4	Băbească gri	842	Băbească neagră-mutant	grey
5	Blasius	20959	(Iordană x Traminer roz) x (Raisin de Saint Pierre x Perla de Csaba)	green yelow
6	Columna	2787	Pinot gris x Grasă de Cotnari	green yelow
7	Crâmpoșie selecționată	3238	Crâmpoşie open pollination	green yelow
8	Donaris	3642	Bicane x Muscat de Hamburg	green yelow
9	Furmint de Miniș	16940	Furmint-mutant	green yelow
10	Miorița	7845	Coarnă neagră open pollination	green yelow
11	Roz de Miniș	10289	Clonal selection from a population of local varieties (probably Bacator roz)	rose
12	Selena	21558	(Iordană x Traminer roz) x (Raisin de Saint Pierre x Perla de Csaba)	rose
13	Șarba	10738	Riesling italian open pollination**	green yelow

**Vitis* International Variety Catalogue; ** Confirmed by markers: Riesling italian x Muscat de Hamburg (Lacombe *et al.,* 2013)

Climatic conditions. For this study, there were used weather data recorded at Bucharest-Baneasa meteorological station for the experimental period (2015-2019), as compared to the reference period (1981-2010). There were studied the following variables: average annual temperature (AAT); average temperature in the growing season (ATGS); average temperature in summer (ATS); average annual minimum temperature (AAT_{min}); absolute minimum temperature (AT_{min}); average annual maximum temperature (AAT_{max}); average of the warmest month's maximum temperatures ($AWMT_{max}$); average maximum temperature in summer (AST_{max}); number of hot days (T_{max} > 30°C); number of very hot days (T_{max} > 35°C); annual total precipitation (ATP); total precipitation in the growing season (TPGS); total precipitation in summer months (TPS). Monthly average temperatures were used to evaluate a set of bioclimatic indices commonly used in viticulture: Huglin index (HI, Huglin, 1978), Winkler index (WI, Winkler et al., 1974) and cool night index (CNI, Tonietto & Carbonneau, 2004).

Phenological data. The four main phenophases (budburst, flowering, veraison, harvesting maturity) were followed, according to BBCH (**B**iologische **B**undesansalt und **C**hemische Industrie), modified under the COST FA 1003

Action (Lorenz et al., 1994; Rustioni et al., 2014b). According to the recorded data, 50% of buds, flowers and grapes reached the respective phenological stages: BBCH 008 (green tips clearly visible) - budburst; BBCH 605 (flowers are open) - flowering; BBCH 805 (changing of berries color, or softening) - veraison and BBCH 809 (Brix according to the cultivar) - berries ripe for ripening.
Each data set was analyzed using variance analysis, One Way ANOVA, post-hoc Tukey HSD $p<0.05$.

RESULTS AND DISCUSSIONS

Changes in climate characteristics of the study area, between 1981-2010 and 2015-2019 time periods

The main climatic parameters of the experimental period compared to the reference period are presented in Table 2. During the studied period (2015-2019), higher temperatures were recorded, as compared to the reference period (1981-2010): the AAT was higher by 0.61°C; the ATGS (IV-X) increased by 0.32°C and $AWMT_{max}$ by 0.31°C. The highest increase (1.26°C) was recorded at average annual maximum temperature (AAT_{max}).
Average temperature for the growing season (17-19°C) includes the Southern part of Romania in warm climate maturity grouping (Jones et al., 2005; White et al., 2006; Ramos et al., 2018; Tomasi et al., 2011; Neethling et al., 2012), similar to some wine region from Italy (Piedmont, Chianti), France (Loire Valley, Bordeaux, Rhone Valley), USA (Margaret River, Northern and Coastal California) etc.
The current average of the ATGS maintains local climate in the *warm* class suitable for the Cabernet Sauvignon, Merlot, Cinsaut varieties (Huglin, 1978), but it approaches by the upper limit of 19°C which indicates the transition to the *hot* climate suitable only for the production of table grapes. Compared with the reference period (1981-2010), during the experimentation period (2015-2019), the number of hot days in the growing season (Tmax > 30°C) increased from 46 to 67, and the number of very hot days (Tmax > 35°C) it doubled, from 6 to 12.2.
Both annual total precipitation and the one in the growing season had a small variation. The increase in ATP by 20 mm in the reference period does not have the potential to balance the increased values of evapotranspiration generated by temperature increases.
Bioclimatic indices (Huglin index, Winkler index and Cool night index) also recorded higher values during the current period, as compared to the reference period, as follows: an increase of 140 units for the Huglin index; an increase of 69 units for the Winkler index; an increase of 1.09°C for the Cool night index. By its increase, the Huglin index passes from the temperate – warm class HI+1 between 1981-2010, to the warm class HI+2 during the recent period. This also changes the climate profile to wich local varieties were adapted and creates the climate context for the growing of new wine grape varieties.
The Winkler index current average maintains in the climate profile specific to Regions III (suitable for high production of standard to good quality wine table wines) but with an increased value approaching climate suitability to that specific to Regions IV (acceptable table wine quality at best). A similar evolution for the CI wich maintains in the class of very cool nights, less suitable to grapes ripening, but with an increased value that reveal the evolution towards the superior cool night class.

Phenology data for the 2015-2019 time-period

The first effect of temperature raising is on advance of phenological stages of grapevine. The differences in the timing of phenological stages and the interval between them are given to genetic factors, climate and soil conditions and viticultural practices (Bucur & Dejeu, 2018). Table 3 shows the average day of the year (DOY) and standard deviations of the four phenological stages of grapevine (budbreak, flowering, veraison and harvest) for the period between 2015 and 2019.
The time of **budbreak** occured on average 106.4 DOY (April 16), earlier for Aromat de Iași and Crâmpoșie selecționată varieties (104.4) and later for Columna variety (110.6). Considering year-to-year variability in budburst, Donaris exhibited the lowest variability (SD ± 2.3 days) while Crâmpoșie selecționată has the greatest variation (SD ± 4.5 days). Standard deviations indicate a moderate interannual variability, ranging from 5 to 9 days.

Flowering occurred on average at DOY 150.1 (May 30), earlier for Crâmpoșie selecționată variety (147.4) and later for Furmint de Miniș (152.0), the differences between varieties being relatively small. Donaris and Roz de Miniș exhibited the least year-to-year variation (SD ± 5.3 days) while Selena had a highest year-to-year variation (SD ± 6.5 days).

Veraison was registered on average at DOY 215.4 (August 03) earlier for Alb aromat variety (211.4) and later for Selena variety (220.0). Roz de Miniș had the lowest year-to-year variation of ± 2.1 days while Columna varied by ± 8.9 days during 2015-2019.

The average day of the year for **grapes ripening**, for the 5 studied years, was the earliest for Aromat de Iași (243.2) and the latest for Roz de Miniș and Selena (251,2), this meaning the 8th of September on average, for the two varieties. Miorița exhibited the lowest year-to-year variability (SD ± 3.4 days) while Alb aromat showed the highest year-to-year vari-ation (SD ±7.6 days). The results concerning the main phenological timing across the growing season shown that there are not always strong relationships between growth events.

Table 2. The main climatic indicators of the experimentation period (2015-2019) compared to the multiannual average (1981-2010)

Climatic parameters and bioclimatic indices	Average 1981-2010	Years 2015	2016	2017	2018	2019	Average 2015-2019	Diference 2015-2019 / 1981-2010
Average annual temperature (AAT), ºC	**11.55**	12.05	11.88	11.74	12.21	12.92	**12.16**	**+ 0.61**
Average temperature in the growing season (ATGS), ºC (IV-X)	**18.07**	17.95	18.13	17.99	19.33	18.53	**18.39**	**+ 0.32**
Average temperature in summer (ATS), ºC (VI-VIII)	**22.50**	2.,3	22.92	22.78	22.83	23.05	**22.78**	+ 0.28
Average annual minimum temperature (AATmin), ºC	**5.03**	5.88	5.78	5.44	6.38	6.57	**6.01**	+ 0.98
Average of absolute minimum temperature, (ATmin), ºC	**-16.99**	-20.40	-19.70	-21.70	-21.70	-15.50	**-19.80**	-1.01
Average annual maximum temperature (AATmax), ºC	**17.05**	18.22	17.98	18.04	18.03	19.28	**18.31**	**+ 1.26**
Average warmest month July maximum temperature (AWMTmax), ºC	**29.87**	3.87	27.76	30.16	28.87	29.64	**29.66**	- 0.21
Average maximum temperature in summer (ASTmax), ºC (VI-VIII)	**29.01**	29.83	30.17	30.01	29.81	30.5	**30.06**	**+ 1.05**
Number of hot days (T_{max} > 30°C)	**46**	62	75	66	71	62	**67**	**+ 21**
Number of very hot days (T_{max} > 35°C)	**6**	23	10	13	6	9	**12.2**	**+ 6.2**
Annual total precipitation (ATP), mm	**608**	632	694	661	623	529	**628**	+ 20
Total precipitation in the growing season (TPGS), mm (IV-X)	**428**	371	514	415	312	385	**400**	- 28
Total precipitation in summer (TPS), mm (VI-VIII)	**198**	150	164	155	228	142	**168**	- 30
Huglin index (HI, Huglin, 1978)	**2346**	2422	2497	2408	2646	2458	**2486**	**+ 140**
Winkler index (WI, Winkler, 1974)	**1726**	1701	1740	1710	1997	1825	**1795**	+ 69
Cool night index (CI, Tonietto and Carbonneau, 2004)	**10.45**	12.6	11,06	12.16	11.30	10.60	**11.54**	+ 1,09

Table 3. Mean day of year (DOY) of the phenological stages (budburst; flowering; veraison and harvest) and the corresponding standard deviations (SD in days), for grapevine varieties for white wine (2015-2019)

Variety	Budburst (50%)	Flowering (50%)	Budburst-Flowering (days)	Veraison (50%)	Flowering-Veraison (days)	Harvest	Veraison-Harvest (days)	Budburst-Harvest (days)
Alb aromat	105.8 ± 4.2^{a}	150.4 ± 6.1^{a}	44.6 ± 6.5a	211.4 ± 5.1^{a}	61.0 ± 4.6a	244.0 ± 7.6a	32.6± 12.5a	138.6 ± 9.1a
Aromat de Iași	104.4 ± 3.2^{a}	148.8 ± 5.8^{a}	44.4 ± 6.7a	213.2 ± 7.8^{a}	64.4 ± 8.7a	243.2 ± 6.8a	30.0 ± 7.6a	138.8 ± 5.3a
Astra	106.8 ± 3.1^{a}	150.6 ± 5.7^{a}	43.8 ± 4.6a	215.0 ± 4.9^{a}	64.4 ± 1.5a	249.8 ± 3.7a	36.4 ± 8.1a	143.0 ± 4.1a
Băbească gri	106.2 ± 3.3^{a}	149.8 ± 5.5^{a}	43.6 ± 5.8a	211.6 ± 4.5^{a}	61.8 ± 2.7a	246.4 ± 5.7a	34.8 ± 8.9a	140.2 ± 8.5a
Blasius	107.2 ± 3.6^{a}	151.6 ± 5.6^{a}	44.4 ± 5.6a	214.4 ± 4.9^{a}	62.8 ± 3.5a	249.8 ± 4.3a	35.4 ± 3.9a	142.6 ± 5.2a
Columna	110.6 ± 4.3^{a}	151.0 ± 6.4^{a}	40.4 ± 5.9a	215.0 ± 8.9^{a}	64.0 ± 6.8a	247.8 ± 3.6a	32.8± 11.4a	137.2 ± 5.6a
Crâmpoșie sel.	104.4 ± 4.5^{a}	147.4 ± 5.4^{a}	43.0 ± 6.6a	219.2 ± 4.6^{a}	71.8 ± 2.8a	250.6 ± 4.3a	31.6 ± 6.2a	146.4 ± 7.6a
Donaris	106.4 ± 2.3^{a}	151.2 ± 5.3^{a}	44.8 ± 5.9a	214.4 ± 6.2^{a}	63.2 ± 1.3a	244.0 ± 7.4a	29.6± 11.7a	137.6 ± 7.1a
Furmint de Miniș	107.0 ± 2.4^{a}	152.0 ± 5.8^{a}	45.0 ± 5.2a	219.4 ± 8.2^{a}	67.4 ± 6.8a	248.2 ± 3.6a	28.4 ± 9.1a	141.2 ± 4.8a
Miorița	107.4 ± 3.8^{a}	151.8 ± 5.8^{a}	44.4 ± 5.6a	217.4 ± 5.0^{a}	65.6 ± 2.5a	248.6 ± 3.4a	31.2 ± 5.3a	142.4 ± 6.6a
Roz de Miniș	107.4 ± 3.4^{a}	151.0 ± 5.3^{a}	43.6 ± 5.7a	219.0 ± 2.1^{a}	68.0 ± 4.1a	251.2 ± 4.3a	32.2 ± 3.9a	143.8 ± 6.4a
Selena	106.4 ± 2.9^{a}	148.6 ± 6.5^{a}	42.2 ± 6.8a	220.0 ± 4.8^{a}	71.4 ± 1.8a	251.2 ± 3.9a	30.8 ± 6.1a	144.4 ± 4.0a
Șarba	105.4 ± 2.8^{a}	149.2 ± 5.8^{a}	43.8 ± 6.3a	213.8 ± 3.8^{a}	64.6 ± 4.6a	246.2 ± 5.6a	32.4 ± 6.5a	140.8 ± 6.4a
Mean	106.4 (April 16)	150.1 (May 30)	43.7	215.4 (August 03)	65.2	247.4 (September 04)	32.1	141.1
Minimal value	104.4	147.4	40.4	211.4	61.0	243.2	28.4	137.2
Maximal value	110.6	152.0	45.0	220.0	71.8	251.2	36.4	146.4

The intervals between phenological events also show some variability, ranged from 40.4 days (Columna) to 45.0 days Furmint de Miniș for budbreak-flowering, from 61.0 days (Alb aromat) to 71.8 days (Crâmpoșie selecționată) for flowering-veraison, from 28.4 days (Furmint de Miniş) to 36.4 days (Astra) for veraison-ripening, and from 137.2 days (Columna) to 146.4 days (Crâmpoșie selecționată) for budbreak-ripening.

As compared to the reference data in literature on the maturity stages (Indreaş & Vişan, 2001; Rotaru, 2009; Stroe, 2021), grapes ripening was anticipated with 5-15 days for medium maturation varieties (Aromat de Iaşi, Donaris, Şarba) and 23-28 days for late maturation (Mioriţa, Crâmpoșie selecționată, Băbească gri, Selena).

Compared with the data presented by Drappier et al. (2019) for Bordeaux vineyard, the data obtained in our study for the interval between flowering and veraison are similar (approximately 65 days), while the veraison-ripening period is lower (on average 32 compared to 45 days).

The lowest intervarietal variability was found in flowering (4.6 days) and the highest one in veraison (8.6 days). Standard deviation (SD) showed the lowest interannual variability in budburst (from 2.3 to 4.5 days) and the highest one in veraison (from 2.1 to 8.9).

In Table 4, the main grape bunch and berry characteristics are presented for the 13 new white grape varieties. Roz de Miniş variety distinguished by the highest value of bunch weight (399.34 ± 24.79 f) and Columna by the minimum value (167.51 ± 22.87c). In most varieties, berries weight, on average, between 2.40 ± 0.25d g/berry (Selena) and 3.23 ± 0.59bd g (Alb aromat). All studied varieties have a spherical berry shape with small deviations, and medium length and width (OIV codes: 220, 221 and 223).

Grape production (kg/vine) was significantly affected by the minimum harmful temperatures in winter (Tmin < -20ºC), especially during 2014/2015 and 2015/2016 periods. The most affected variety was Columna (2.13 ± 1.33a kg/vine).

Warmer climate conditions are associated with increased sugar accumulation and decreased titratable acidity. As for the sugar concentration (ºBrix), high levels are found in most varieties (Table 5). Significant maximum sugar accumulations were recorded to Aromat de Iași (23.33 ± 0.88ºBrix), followed by Şarba (23.06 ± 0.87ºBrix) and minimum sugar accumulations to varieties Astra (19.38 ± 1.29ºBrix) and Columna (19.98 ± 0.79ºBrix).

The highest titratable acidity concentration was registered in Aromat de Iași (7.81 ± 0.68 $g.L^{-1}$ acid tartric) and the lowest in Roz de Miniș (5.33 ± 0.76 $g.L^{-1}$ acid tartric).

In order to appreciate the optimal grape maturity, it is important to calculate glucoacidimetric index and to compare it with optimal values (Shellie, 2007; Irimia, 2012). Most varieties are closer to optimum maturity. The qualitative characteristics of most studied varieties showed the possibility to obtain good quality wines, starting from a sugar content higher than 21 ºBrix.

Table 4. Grapes quantitative parameters at harvesting time for grapevine varieties for white wine* (2015-2019)

Crt. no.	Variety	Bunch weight (g)	Berry weight (g)	Yield (kg / vine)
1	Alb aromat	288.39 ± 35.45 b	3.23 ± 0.59 bd	3.00 ± 1.24 a
2	Aromat de Iași	191.46 ± 22.12 c	2.81 ± 0.40 bc	3.20 ± 0.74 a
3	Astra	217.57 ± 12.46 cd	3.09 ± 0.30 bc	2.44 ± 0.75 a
4	Băbească gri	186.69 ± 14.24 c	2.85 ± 0.32 bcd	2.29 ± 0.98 a
5	Blasius	185.39 ± 39.55 c	3.17 ± 0.67 b	3.08 ± 1.30 a
6	Columna	167.51 ± 22.87 c	2.59 ± 0.18 cd	2.13 ± 1.33 a
7	Crâmpoșie selecționată	220.04 ± 31.97 ce	3.08 ± 0.25 b	3.12 ± 0.70 a
8	Donaris	195.97 ± 22.08 c	3.13 ± 0.24 b	2.49 ± 0.92 a
9	Furmint de Miniș	203.65 ± 40.33 c	3.05 ± 0.20 bc	2.83 ± 1.22 a
10	Miorița	206.89 ± 38.62 ce	2.92 ± 0.45 bc	2.73 ± 1.29 a
11	Roz de Miniș	399.34 ± 24.79 f	3.17 ± 0.35 b	2.62 ± 0.73 a
12	Selena	189.92 ± 37.89 c	2.40 ± 0.25 d	2.78 ± 1.10 a
13	Șarba	184.86 ± 50.64 c	2.76 ± 0.58 bcd	2.62 ± 0.88 a
	Minimal value	167.51	2.40	2.13
	Maximal value	399.34	3.23	3.20

Table 5. Qualitative parameters at harvesting time for grapevine varieties for white wine* (2015-2019)

Crt. no.	Variety	Sugar content (ºBrix)	Titratable acidity ($g.L^{-1}$ tartaric acid)	Glucoacidimetric index
1	Alb aromat	20.45 ± 0.89 ^{ab}	5.52 ± 0.63 c	37.52 ± 4.59 c
2	Aromat de Iași	23.33 ± 0.88 cb	7.81 ± 0.68 a	30.10 ± 3.14 b
3	Astra	19.38 ± 1.29 a	7.78 ± 0.61 a	25.00 ± 2.05 bd
4	Băbească gri	20.87 ± 1.02 b	7.12 ± 0.71 a	29.66 ± 4.01 b
5	Blasius	20.93 ± 0.86 b	7.79 ± 0.46 a	27.00 ± 2.57 bd
6	Columna	19.98 ± 0.79 a	6.51 ± 0.32 ca	30.80 ± 2.32 b
7	Crâmpoșie selecționată	21.97 ± 1.33 cb	6.78 ± 0.76 ca	32.83 ± 4.58 bc
8	Donaris	21.92 ± 1.08 cb	6.77 ± 0.67 ca	32.80 ± 4.57 bc
9	Furmint de Miniș	21.77 ± 0.92 cb	7.50 ± 0.77 a	29.34 ± 3.56 b
10	Miorița	20.03 ± 1.22 a	7.18 ± 0.47 a	28.07 ± 2.97 b
11	Roz de Miniș	21.05 ± 1.42 b	5.33 ± 0.76 b	40.46 ± 7.42 c
12	Selena	21.83 ± 1.11 b	7.21 ± 1.00 a	30.95 ± 5.46 b
13	Șarba	23.06 ± 0.87 c	6.97 ± 1.21 a	35.39 ± 6.93 b
	Minimal value	19.38	5.33	25.00
	Maximal value	23.33	7.81	40.46

*Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for grape varieties for p<0.05. For the same compound, a common letter for 2 or more variants shows no significant difference among them; One Way ANOVA, post-hoc Tukey HSD p<0.05.

Comparing the qualitative parameters obtained in this study with those reported in Ampelography, vol. IX for the period 1980-2000, differences can be observed. Thus, during the experimental period (2015-2019) an anticipation of the grape ripening period was found by approximately 15 days (for the Aromat de Iași, Columna, Furmint de Miniș varieties) and up to 30 days (for the Băbească gri, Crâmpoșie selecționată, Roz de Miniș and Selena) (Table 6).

Increased accumulations of sugars were also recorded, between + 14 g/L (Şarba) and + 33 g/L (Aromat de Iasi), compared to the period 1980-2000. In most varieties the titratable acidity recorded lower values. The optimal values of the glucoacidimetric index are between 30-50, for the varieties Alb aromat, Aromat de Iași, Columna, Donaris, Roz de Miniș, Selena and Şarba (Table 6).

Table 6. The main characteristics of the new varieties for white wines obtained in the experimental period (2015-2019), compared to the reference period (1980-2000)

Crt. no.	Variety	Period of grapes maturation		Sugar content (ºBrix)		Titratable acidity (g.L^{-1} tartaric acid)		Glucoacidimetric index	
		1980-2000*	2015-2019	1980-2000*	2015-2019	1980-2000*	2015-2019	1980-2000*	2015-2019
1	Alb aromat	IV-V	IV	19.7-22.0	20.5	5.4-6.9	5.52	37-32	37
2	Aromat de Iași	IV	III	17.0-20.0	23.3	6.1-6.7	7.81	43-30	30
3	Astra	V-VI	**IV**	16.4-20.4	19.4	9.2-9.9	7.78	18-21	25
4	Băbească gri	VI	**IV**	16.8-19.5	20.9	9.2-10.3	7.12	18-19	29
5	Blasius	V-VI	**IV**	19.6-20.0	20.9	8.9-10.5	7.79	22-19	27
6	Columna	V	IV	18.4-22.0	20.0	7.7-9.2	6.51	24-23	31
7	Crâmpoșie sel.	VI	**IV**	18.0-20.6	21.9	6.4-7.0	6.78	28-29	32
8	Donaris	IV	IV	18.0-22.0	21.9	7.7-8.6	6.77	23-26	32
9	Furmint de Miniș	V	IV	18.0-19.5	21.8	6.1-8.7	7.50	29-22	29
10	Miorița	V-VI	**IV**	16.5-18.0	20.0	8.6-11.5	7.18	19-16	28
11	Roz de Miniș	VI	**IV**	13.6-18.2	21.1	5.5-6.6	5.33	25-28	39
12	Selena	VI	**IV**	18.8-21.0	21.8	8.1-9.2	7.21	23-30	30
13	Șarba	V	IV	18.7-21.7	23.1	7.5-7.9	6.97	25-27	33

*after Ampelography, vol. IX.

CONCLUSIONS

The study has shown that climate warming influences, to a greater or lesser extent, all the 13 new varieties for white wines.

During the experimentation period (2015-2019) an increase of the average annual temperature with 0.61ºC was observed, and of the average maximum summer temperature with 1.05ºC, compared to the reference period (1981-2010). There was also a marked increase in heat waves, represented by the number of days with $T_{max} > 30$ºC and $T_{max} > 35$ºC. In the last five years was an increase of 140 units for the Huglin index which passes from temperate - warm class (HI + 1) to the warm class (HI + 2).

There have been significant changes in grapevine phenology, as well as in the quantity and quality of grape production.

The highest differences compared to reference period, were recorded at grape ripening, the anticipation being between 11.5-14.3 days. These differences were due to the increase of the maximum temperatures in the summer season, with 1.05ºC.

The average length of the budbreak-harvest cycle for the studied period (2015-2019) and for all varieties was shortened, reaching 137.2 to 146.4 days. The longest phenological cycle (from budburst to grapes ripening) were observed at Crâmposie selecționată variety (146.4 days), due to early budburst and late maturation.

The best behavior under quantitative terms, but mainly under qualitative ones, was found in Aromat de Iaşi, Şarba, Crâmposie selecționată and Băbească gri varieties.

Considering the anticipation of a more intense climate warming for the future (IPCC, 2018), it is necessary to promote varieties with moderate accumulations of sugars, with late grape maturation, in order to avoid ripening during excessive temperature periods.

The exploration of the genetic capacity of the different new varieties of grapevine under the current conditions, provides an important means of adaptation to the climatic warming. The recorded data in our study allow understanding the local diversity of new grapevine varieties for white wines, which is an important step to explore the phenotypic diversity among grapevine varieties.

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RESEARCH ON INCREASING THE ADAPTATION CAPACITY OF GRAPEVINE TO CLIMATE CHANGE - TREATMENTS WITH KAOLIN AND ZEOLITES

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Abstract

Climate change, global warming with the increase of thermohydric stress during the ripening of the berries, the greater frequency of extreme climatic phenomena, etc., produce physiological and biochemical changes in the growth and fruiting of the vine, influencing the production of grapes and especially its quality. The experience carried out in 2022 and 2023 on the 'Fetească regală', under the conditions of the experimental plantation of the UASVM Bucharest Romania, aimed at mitigating the effects of summer thermohydric stress, by applying treatments with kaolin and zeolite in concentrations of 3 and 5 %. After applying the treatment, the intensity of photosynthesis and transpiration was determined, the quantity and quality of the harvest. The variant zeolite 5% stood out, where intensity of photosynthesis increased by 7.6 µmol CO_2 m^{-2} s^{-1} in 2022 and 4.45 µmol CO_2 m^{-2} s^{-1} in 2023, compared to the control; and the transpiration rate decreased by 2.24 mmol H_2O m^{-2} s^{-1} compared to the control, in 2022, for the zeolite 3% variant. Also, the concentration of sugars decreased by 3.67ºBrix in 2022 and 1.66 in 2023, for the zeolite 3 and 5% variants.

Key words: *climate change, foliar application, heat waves, kaolin, zeolites*

INTRODUCTION

According to projections by the Intergovernmental Panel on Climate Change (IPCC, 2014), the global average surface temperature of the Earth is likely to increase this century by between 1.8 and 4.0°C. In this context, it is important to implement innovative measures to mitigate and combat the negative effects of climate change.

Grapevines are one of the plants most affected by these changes, being increasingly subject to radiation, heat and water stress, with negative effects on production and especially its quality.

Numerous studies over the years have established the optimal temperature values for photosynthesis and sugar accumulation in berries (25-30°C) and for anthocyanin accumulation (17-26°C). Lower values are also favourable for the accumulation of aromatic compounds (Mori et al., 2007; Tarara et al., 2008). Research carried out in Australia by Coombe (1987) established that temperatures between 25 and 28°C provide an optimum ratio of sugars to anthocyanins.

Exceeding these intervals, due to global warming, especially during the grape ripening period, and heat waves have led to a series of negative reactions: very high accumulation of sugars in the berries, with marked degradation of acidity, increased pH values, development of atypical aromatic compounds, the phenolic maturity being also affected. Thus, the resulting wines are less suitable for maturation, have a modified aromatic profile and a weaker colour (Palliotti et al., 2015; Martinez de Toda & Balda, 2015; van Leeuwen et al., 2019; Reynold, 2021).

At all meteorological stations in Romania was found an increase in the average annual temperature and a shortening of the transitional seasons (spring and autumn), which indicates a tendency for temperate zones to move closer to subtropical climate conditions.

In most of the country's vineyards, studies have shown significant warming with an influence on the development of the phenological stages of grapevine, the main physiological processes, vegetative growth, grape production and quality (Enache & Donici, 2014; Irimia et al., 2015; Dobrei et al., 2017; Bucur et al., 2019; Cichi et al., 2019; Stroe & Cojanu, 2019).

Șerdinescu et al. (2014), found an increase in mean air temperature between 2000 and 2010 in the main vineyards of the country, with values ranging from 0.7 to 2.1ºC. The largest differences were recorded in Dealu Mare, Târgu Bujor and Murfatlar vineyards highlighting their aridization trend.

Research carried out in 7 centres located in different regions of the country, over a period of 40 years (1977-2016), (Bucur & Dejeu, 2016) found significant increases in mean annual temperatures between from 1.2ºC (Bucharest) to 2.5ºC (Cluj Napoca). Similar increases (1.2-2.3ºC) were recorded during the growing season of grapevines (April-October), while in the hottest month of the year (July), the maximum temperature increases were much higher (3.2-5.5ºC).

Heat waves, assessed on the basis of the number of days with maximum temperatures above 30ºC and 35ºC (the heat wave threshold), showed a distinct statistically significant upward trend in all 7 centres studied in the country (Bucur & Babeș, 2016). Increasing the number of days with Tmax > 35ºC determined a reduction in grape production, average berry mass, a decrease in acidity and an increase in the concentration of sugars in the berry. Due to the intense solar radiation and extreme temperature values during the ripening period of the grapes (4 consecutive days with Tmax > 40ºC), sunburn was recorded on the grapes on the western side of the rows oriented in the N-S direction. Under these conditions, the application of shoot topping and the defoliation with the removal of part of the most photosynthetically active leaves, to the 'Fetească regală' in 2017-2019, led to statistically significant results, reducing these shortcomings, through lower sugar accumulation (by 1.3ºBrix), maintaining acidity in normal parameters (Bucur, 2021).

To mitigate the negative effects, viticulture has a series of **innovative measures** to delay the ripening of grapes and obtain balanced wines with an average alcohol content, namely: *short-term measures* - soil maintenance (Dhanush & Patil, 2020; Buesa et al., 2021), plant management (Palliotti et al., 2014; Silvestroni, et al., 2019), choice of harvest time, winemaking techniques; *in the medium term* - the orientation of the rows, the choice of land for planting, the use of suitable rootstocks (Carvalho et al., 2020) and *in the long term* - the use of irrigation, even late, obtaining new varieties more adapted to these conditions (Caccavello et al., 2019; Miras-Avalos & Araujo, 2021).

Short-term strategies are of particular importance, especially interventions on the foliar apparatus (severe shoot topping, defoliation of the stumps with the removal of part of the most photosynthetically active leaves, applied to the grape veraison and treatments with natural products that protect plants from the effects of radiation and thermohydric stress - kaolin and zeolite).

The use of substances with antiperspirant action causes a partial closing of the stomata, a reduction of photosynthesis and water loss through transpiration.

Spraying plants with kaolin, a natural clay product, has been shown to be effective by forming a film on the surface of the leaves to reflect light and protect against heat stress and water deficit.

By foliar application of a repeated **kaolin** treatment (5%), immediately after the grapes veraison, an increase of 40% in total phenolics, 24% in flavonoids and 32% in anthocyanins was obtained in Touriga National cultivated in the Douro region (Portugal) under conditions of heat and water stress (Dinis et al., 2016; Valentini et al., 2021). Applying a treatment to the Pinot noir variety with kaolin (3%), on the western side of the row, at the beginning of August caused a reduction in leaf temperature by 4-6ºC compared to the control, ultimately leading to a 27% increase in production, a anthocyanin content by 35% and acidity by 11%. The observations were made during the 2017 heat wave in the Umbria region of Italy (Frioni et al., 2019; Singh et al., 2020). Together with kaolin, the use of **zeolite** is a viable and innovative alternative in the sustainable approach to mitigating the effects of global warming and heat waves during the grape ripening period, as well as in the field of disease and pest control allowed in organic farming. Natural zeolite is a sedimentary rock of volcanic origin, composed of hydrated aluminosilicates of calcium, sodium, potassium, magnesium, manganese etc. They used zeolites to control gray rot (*Botrytis cinerea*) and grape moth (*Lobesia botrana*) compared to the application of synthetic fungicides and insecticides in the Abruzzo region of Italy (Calzarano et al., 2019).

The results were comparable to those obtained in the case of the use of synthetic pesticides, without affecting the production and quality of the grapes (accumulation of sugars, titratable acidity and pH). In addition, the application of zeolites led to an increase in the concentration of anthocyanins and an improvement in the color of the wines. This result is similar to that of foliar application of kaolin, which is an inert, radiation-reflecting mineral capable of reducing leaf temperature and influencing major secondary metabolic pathways leading to the biosynthesis of anthocyanins, phenolic and flavor compounds.

This study proposed to analyse the effect of the application of two natural antitranspirant substances (kaolin and zeolite, in different concentrations), on certain physiological parameters and the composition of grapes in the 'Fetească regală'.

MATERIALS AND METHODS

Experimental conditions and the biological material

The research was carried out during the vegetation period related to the years 2022 and 2023, two dry years in which numerous heat waves were recorded, in the experimental plantation within the UASMV Bucharest (N Lat.: 44° 47' 07"; E Long .: 26° 07' 28"; alt. 87 m), on the variety to 'Fetească regală' clone 21 Bl, grafted on Kober 5 BB rootstock. The vine planting distance is 2.2 m x 1.2 m, resulting in a density of 3788 plant ha-1, and the pruning type is bilateral cordon with spur pruning system and loading of 12 buds/m^2.

The vines is grown in a conventional system, without irrigation aid 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 and on the row of vines by herbicide.

The experimental variants

The experimental design included 100 vines distributed in five randomized blocks in three different rows. Each block consisted of 20 vines, on which foliar treatments with kaolin and zeolite were applied in different concentrations:

→ Control - untreated control vines;

→ KAO - spray treatment with kaolin, in 3% and 5% concentration;

→ ZEO - foliar treatment with Romanian natural zeolite, in 3% and 5% concentration.

The suspensions were carefully sprayed on both sides of the canopy using a backpack sprayer (Figure 1), once, shortly after entering the veraison phase (August 05 - DOY 217, în anul 2022 si pe August 03 - DOY 215, în 2023). A guard row was left between the experimental variants.

Parameters determined

▪ *Leaf gas exchange measurements*

Photosynthesis intensity (A) (µmols CO_2 $m^{-2}s^{-1}$) and transpiration intensity (E) (mmol H_2O $m^{-2}s^{-1}$) were determined on 12 and 19 August (2022) and 11 and 18 August (2023) between 10:00 and 13:00, respectively. On each vine, 3 replicates (three mature, healthy leaves between nodes 6-7 were analysed in each replicate), using a BioScientific ADC LCpro-SD portable photosynthesis system equipped with an infrared gas analyser. Leaves were enclosed in the chamber (under ambient conditions), held for 2 min for acclimation, then 5 readings per repetition were recorded at 1 minute intervals.

▪ *Yield components and berry composition*

The grapes were harvesting on the date of September 01, 2022 (DOY 244), respectively September 13 in 2023 (DOY 256).

At harvesting, for control and each experimental variant, determinations were made on quantitative (grape weight – grams, berry weight – grams, yield – kg/vine) and qualitative parameters (sugar content – ºBrix, titratable acidity – g/L tartaric acid). 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.

Figure 1. Aspects from the plantation, following the application of treatments with kaolin and zeolite

Statistical analysis

Data were processed using Microsoft Excel (version 2010) and are shown as average values ± Standard Error (SE). 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 between different experimental variants or the periods. Statistically significant differences have been considered at the value of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Evolution of environmental conditions in years 2022 and 2023

Table 1 shows the evolution of the most important climate parameters for the experimental period (2022-2023) compared to the reference period (1991-2020), after the recommendations of National Meteorological Administration (Dima et al., 2019).

A careful analysis of them, during the two years of the study, showed that 2023 was a warmer and less rainy year than 2022. In particular, there were differences of 3.15°C in the case of average temperature in summer (VI-VIII), of 2.06°C for average temperature in the growing season (IV-X), of 1.62°C for average annual temperature, 1.4°C for average annual maximum temperature and 0.96°C for average temperature in the warmest month. At the same time, there was a decrease in precipitation during the vegetation period, by 64.5 mm in 2023, compared to 2022.

Comparing the average of the years in which the experience was carried out with the multiannual average over 30 years, it can be seen that all the values of the climatic parameters have registered large increases in the last two years, as follows: average annual temperature by 2.82ºC, average temperature in the growing season (IV-X) with 2.52ºC, average temperature in summer (VI-VIII) with 3.5ºC, average annual maximum temperature with 2.9ºC, average temperature in the warmest month with 3.16 ºC etc. Also noteworthy is the evolution of Tmax > 30°C and Tmax > 35°C where the number of days increased by 6 and 13 days, respectively, in favor of the last two years. The Huglin heliothermal index reached an average value of 3802 units during the experimental period, exceeding by 639 units the value recorded in the reference period, 1991-2020, respectively 3163, placing the area in ***the very warm climate class.***

Table 1. The main climatic parameters and bioclimatic indices during the experimentation period (2022-2023) compared to the reference period (1991-2020)

Climatic parameters and bioclimatic indices	Average 1991-2020	Years 2022	Years 2023	Average 2022-2023
Average annual temperature, ºC	10.98	12.99	14.61	13.80
Average temperature in the growing season (IV-X), ºC	17.46	18.95	21.01	19.98
Average temperature in summer (VI-VIII), ºC	22.15	24.07	27.22	25.65
Average annual maximum temperature, ºC	17.6	19.8	21.2	20.50
Average temperature in the warmest month, ºC	30.26	32.94	33.9	33.42
Average maximum temperature in summer (VI-VIII), ºC	29.68	32.03	32.86	32.45
Number of hot days (T_{max} > 30°C)	48	53	54	54
Number of very hot days (T_{max} > 35°C)	10	21	25	23
Annual total precipitation, mm	633	384	435	410
Total precipitation in the growing season (IV-X), mm	429.9	281.0	216.5	248.8
Total precipitation in summer (VI-VIII), mm	193.4	90.4	93.7	92.1
Hydrothermal coefficient (CH)	1.1	0.77	0.51	0.64
Huglin index (HI)	3163	3550	4054	3802
Winkler index (WI)	1762	1898	2356	2127
Cool night index (CI)	10.64	10.43	14.10	12.27

Values higher than 2400 units of the Huglin index lead to more unbalanced wines with higher alcohol concentration, sometimes over 13.5% vol. alcohol and the mandatory need for acidity corrections (Bucur et al., 2019).
This evolution of climate parameters confirms a series of researches carried out recently, which have highlighted the harmful effect of heat waves on the vine in the southern part of Romania (Mereanu, 2010; Bucur & Babeș, 2016, Bucur 2021), respectively, changes on the leaf apparatus and phenomena of wilting of the berries (Figures 2 and 3).
Hence the need to protect this perennial plant exposed to radiation and thermal stress.

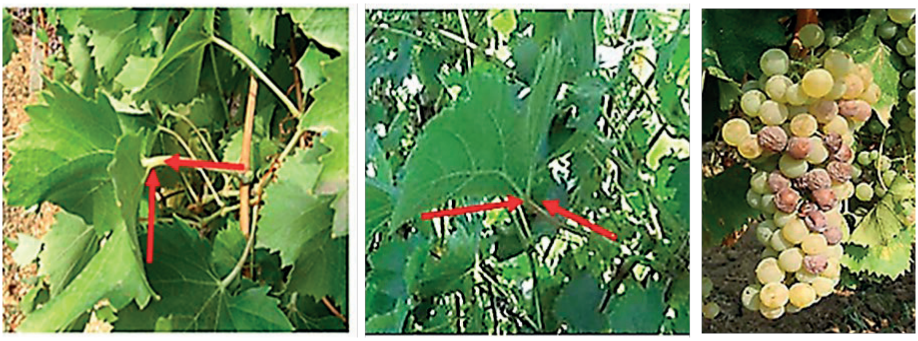

Figure 2. The change induced by heat waves, solar radiation and water stress on the angle of insertion of the limb of the leaf on the petiole (A) compared to the normal situation (B) and detail with sunburned berries, 26.08.2007 (after Mereanu, 2010)

Figure 3. Sunburn and wilting of the berries, after a summer heat wave registered in August 2022

Leaf gas exchange measurements
The obtained results as regard as of the leaf level gas exchanges and leaf water use efficiency are shown in Table 2. Analyzing the results obtained between the treatments applied on August 12, 2022, the photosynthesis intensity had the higher in the case ZEO 5% (13.85 μmol CO_2 m^{-2} s^{-1}), followed by KAO 5% (7.46 μmol CO_2 m^{-2} s^{-1}), respectively ZEO 3% (7.20 μmol CO_2 m^{-2} s^{-1}). Values are significantly lower between control, KAO 5% and ZEO 3%. The lowest photosynthesis rate has been registered for KAO 3% (3.29 μmol CO_2 m^{-2} s^{-1}), the differences being statistically significant as against KAO 5%, ZEO 3% and 5%; instead the differences are statistically insignificant if we compare with the control. And in the second determination, from August 19, 2022, the ZEO 5% variant reached the highest value (21.38 μmol CO_2 m^{-2} s^{-1}) for net photosynthesis. Significant differences in leaf photosynthesis rate during 2023 were found, for ZEO 5% (9.23 μmol CO_2 m^{-2} s^{-1}).
Regarding transpiration rate, the lowest values were recorded for the ZEO 3% variant (2.56 and 4.20 mmol H_2O m^{-2} s^{-1}) in 2022; respectively to the variants ZEO 3% (1.05 mmol H_2O m^{-2} s^{-1}) and KAO 5% (0.41 mmol H_2O m^{-2} s^{-1}), in the year 2023. Treatments with kaolin and zeolite applied in 2022 led to a decrease in

transpiration rate, compared to the control, in all experimental variants, which shows that the film distributed on the plant apparatus caused a decrease in the temperature of the leaves that lasted over time.

The treatment with zeolite in a concentration of 5%, which stood out for the highest values obtained by net photosynthesis, also reached increased values for transpiration rate; in this case the transport of water and mineral salts is more accentuated at higher values of the transpiration rate.

The synthetic water use efficiency (WUE) parameter recorded the highest values in 2022 for the zeolite treatments (3.24 - ZEO 5% and 5.09 - ZEO 3%), and in 2023 for the ZEO 3% variants - 8.19, respectively KAO 3% - 7.29.

Yield components and berry composition

Table 3 show to 'Fetească regală' berry composition among the treatments aplication (KAO 3%, KAO 5%, ZEO 3%, ZEO 5% and control) in terms of technological maturity (grape weight, berry weight, yield, sugar content and titrable acidity).

Grape weight and berry mean weight per vine was not significantly different between treatments, nor were yield. The drought conditions observed in this study limited the potential yield of the control, which was not different to experimental variants, treatments with kaolin and zeolite.

Regarding the accumulation of sugars in the berries, in the 2022 year of experimentation, a significant reduction in sugar content was found, on average from 26.37°Brix in the control, to 22.70°Brix in the ZEO 3% variant and 23.43°Brix for ZEO 5%. In 2023 there were no significant differences between the treatments in terms of the accumulation of sugars.

Table 2. The leaf net photosynthesis (A), transpiration intensity (E) and water use efficiency (WUE) during the 2022 and 2023 seasons to 'Fetească regală'

Treatment	12 August 2022	19 August 2022	11 August 2023	18 August 2023	Average treatment 1 (2022-2023)	Average treatment 2 (2022-2023)
A - Net photosynthesis (µmol CO_2 m^{-2} s^{-1})						
Control	6.25 ± 1.24 bcC	18.12 ± 1.68 aA	4.78 ± 0.09 bC	1.89 ± 0.98 aD	5.52 ± 0.66 bcC	10.00 ± 0.87 bcB
KAO 3%	3.29 ± 0.21 cC	13.71 ± 1.76 bA	4.47 ± 0.76 bC	4.65 ± 2.16 aC	3.88 ± 0.46 cC	9.18 ± 1.31 bcB
KAO 5%	7.46 ± 1.06 bB	12.61 ± 1.25 bA	4.74 ± 0.59 bC	1.55 ± 0.15 aD	6.10 ± 0.48 bcBC	7.08 ± 0.64 cBC
ZEO 3%	7.20 ± 0.04 bB	21.06 ± 0.36 aA	8.01 ± 0.72 aB	2.51 ± 0.38 aB	7.60 ± 0.34 bB	11.78 ± 0.14 abB
ZEO 5%	13.85 ± 1.82 aB	21.38 ± 1.52 aA	9.23 ± 1.17 aBC	5.21 ± 1.98 aC	11.54 ± 1.48 aB	13.30 ± 0.93 aB
E - Transpiration rate (mmol H_2O m^{-2} s^{-1})						
Control	4.80 ± 0.57 aB	5.75 ± 0.40 aA	1.55 ± 0.12 abBC	0.50 ± 0.22 aC	3.18 ± 0.24 aAB	3.17 ± 0.10 aAB
KAO 3%	3.00 ± 1.83 aAB	4.45 ± 0.39 aA	1.94 ± 0.41 abAB	1.11 ± 0.53 aB	2.47 ± 1.01 aAB	2.78 ± 0.25 aAB
KAO 5%	3.17 ± 0.62 aB	5.49 ± 0.49a A	1.70 ± 0.08 abC	0.41 ± 0.08 aD	2.44 ± 0.33 aBC	2.95 ± 0.28 aB
ZEO 3%	2.56 ± 0.74 aB	4.20 ± 0.38 aA	1.05 ± 0.24 bC	1.02 ± 0.43 aC	1.81 ± 0.30 aBC	2.61 ± 0.05 aB
ZEO 5%	4.60 ± 1.17 aAB	5.60 ± 0.45 aA	2.66 ± 0.47a BC	1.27 ± 0.78 aC	3.63 ± 0.82 aABC	3.43 ± 0.54 aBC
WUE - Water use efficiency (A/E)						
Control	1.47 ± 0.29 aB	3.52 ± 0.33 abAB	3.32 ± 0.28 bAB	3.75 ± 1.10 aA	2.39 ± 0.12 bAB	3.63 ± 0.57 abAB
KAO 3%	2.05 ± 0.78 aB	3.06 ± 0.14 bAB	2.36 ± 0.16 bB	7.29 ± 3.29 aA	2.20 ± 0.39 bB	5.17 ± 1.70 abAB
KAO 5%	2.49 ± 0.51 aA	2.37 ± 0.43 bA	2.76 ± 0.22 bA	4.29 ± 1.31 aA	2.63 ± 0.15 bA	3.33 ± 0.84 bA
ZEO 3%	3.23 ± 0.71 aB	5.09 ± 0.43 aB	8.19 ± 1.17 aA	4.96 ± 1.29 aB	5.71 ± 0.50 aB	5.03 ± 0.86 aB
ZEO 5%	3.24 ± 0.48 aB	3.87 ± 0.41 abAB	3.53 ± 0.21 bAB	6.24 ± 1.94 aA	3.38 ± 0.35 abAB	5.05 ± 1.04 aAB

Legend: KAO (Kaolin), ZEO (Zeolite). Data are shown as mean value ± SE (N = 3). The comparison was made by column between the different experimental variants and on the row between periods when physiological parameters were determined, by Duncan post-hoc Multiple Range Test (DMRT) by using IBM SPSS Statistics software. Statistically significant differences have been considered at the value of $p \leq 0.05$ and are represented by different letters (lowercase - comparison of experimental variants and uppercase - comparison of periods).

The accumulation of sugars in grape berries is influenced by several factors other than the climatic conditions during the ripening period of the grapes, namely: variety, the buds load attributed to pruning of vine, green work and operations applied and soil fertility.

Titratable acidity was maintained at normal values (6.6-6.9 g/L tartaric acid) in the case of treatments with kaolin 3% and 5%, but following the application of treatment with zeolite 5% the acidity took on a higher value (7.94 g/L tartaric acid), higher than the control.

Table 3. Yield parameter and grape components recorded in 'Fetească regală' treated with kaolin, zeolite and to the control vines in 2022 and 2023

Treatment	Grape weight (g)		Berry weight (g)		Yield (kg/vine)		Soluble solids (°Brix)		Total acidity (g/L tartaric acid)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Control	81.80 ± 3.73 a	91.09 ± 2.31 a	1.79 ± 0.07 b	1.60 ± 0.08 a	3.20 ± 0.19 a	2.88 ± 0.23 a	26.37 ± 0.15 a	22.83 ± 0.20 a	5.35 ± 0.19 a	6.15 ± 0.01 b
KAO 3%	71.15 ± 10.6 a	87.17 ± 3.42 a	1.84 ± 0.02 ab	1.78 ± 0.14 a	3.38 ± 0.19 a	2.50 ± 0.29 a	25.67 ± 0.23 b	22.17 ± 1.22 a	5.13 ± 0.1 a	6.95 ± 0.21 ab
KAO 5%	75.35 ± 6.92 a	83.95 ± 2.71 a	1.73 ± 0.07 b	1.63 ± 0.14 a	3.45 ± 0.11 a	2.81 ± 0.18 a	24.57 ± 0.18 c	21.27 ± 1.02 a	5.15 ± 0.28 a	6.99 ± 0.51 ab
ZEO 3%	78.00 ± 5.19 a	79.22 ± 6.69 a	1.88 ± 0.06 ab	1.44 ± 0.12 a	2.98 ± 0.11 a	2.55 ± 0.13 a	22.70 ± 0.31 e	21.87 ± 1.25 a	5.20 ± 0.07 a	6.41± 0.19 b
ZEO 5%	77.30 ± 7.38 a	86.92 ± 5.62 a	1.99 ± 0.06 a	1.66 ± 0.11 a	3.81 ± 0.46 a	2.63 ± 0.09 a	23.43 ± 0.12 d	21.17 ± 0.32 a	5.11 ± 0.07 a	7.94 ± 0.47 a

Legend: KAO (Kaolin), ZEO (Zeolite). Data are shown as mean value ± SE (N = 3). The comparison was done on columns between the different experimental variants, by the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software. Statistically significant differences have been considered at the value of $p \leq 0.05$ and are represented by letters lowercase.

CONCLUSIONS

Considering the future amplification of climate changes, with a negative influence on the vine, it is necessary to intervene with a series of mitigation measures in the short term, with immediate effect. Among these measures is the **application of sun protection substances**, with natural antiperspirant products based on clay (kaolin and zeolite).

The application of treatments with kaolin and zeolite in different concentrations (3 and 5%), at the beginning of the grape ripening phenophase, significantly improved photosynthesis and reduced the intensity of transpiration, which led to an intrinsic efficiency of water use in the vines in the field conditions at the time of the measurements.

Following the observations and determinations carried out over two years, the experimental variant ZEO 5% stood out, which recorded an increase in the rate of photosynthesis both in 2022 (13.85 µmol CO_2 m^{-2} s^{-1} August 12 and 21.38 µmol CO_2 m^{-2} s^{-1} August 19), but also in 2023 (9.23 µmol CO_2 m^{-2} s^{-1} August 11 and 5.21 µmol CO_2 m^{-2} s^{-1} August 18), compared to the control.

Regarding transpiration rate, the lowest values were recorded for the ZEO 3% variant (2.56 and 4.20 mmol H_2O m^{-2} s^{-1}) in 2022; respectively to the variants ZEO 3% (1.05 mmol H_2O m^{-2} s^{-1}) and KAO 5% (0.41 mmol H_2O m^{-2} s^{-1}), in the year 2023.

From an economic point of view, the application of these treatments does not require additional costs, compared to conventional technology, kaolin and zeolites are accessible materials, the use of which does not require equipment or expensive investments, and the spectrum of use is wider, ensuring phytosanitary protection.

The obtained results are of great interest, because they allow a better understanding of the effect of these antiperspirant treatments as a measure to protect the vine from radiation, thermal and water stress, with negative effects on the plant apparatus, its production and quality.

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CARBOHYDRATE DYNAMICS IN SOME GRAPEVINE (*VITIS VINIFERA* L.) CULTIVARS DURING DORMANCY

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Abstract

The adaptation and resistance of the vine to low temperatures during the dormancy involves the realization of processes of accumulation and dynamics of some biochemical compounds in the tissues. In this study, between November and March (2022-2023), the dynamics of carbohydrates (soluble sugars and starch) was monitored at 10 days in the annual and multiannual vine wood in the 'Cabernet Sauvignon', 'Fetească neagră' and 'Merlot' from the Banu Mărăcine wine-growing center (plateau-type area, slightly leached reddish-brown soil) and Șimnicu de Sus wine-growing area (slope-type area, medium eroded reddish-brown soil). Regarding the carbohydrate content during the dormancy, significant differences between the two viticultural areas were highlighted only concerning the content of soluble sugars (%) in multiannual wood for the 'Merlot' and 'Cabernet Sauvignon'. As for the starch content (%) in annual woody shoots and in multiannual wood, no statistically significant differences were observed either between the studied cultivars or between the two areas.

Key words: *carbohydrate dynamics, freezing tolerance, dormancy.*

INTRODUCTION

Carbohydrates, a group of substances of particular importance for vines, are produced by photosynthesis and represent some of the most important elements that form the biochemical composition of the vine. They are the building blocks of organic compounds, store energy, and form support structures, such as cellulose, hemi-cellulose, and gluco-protein (Zufferey et al., 2012; De Rosa et al., 2022).

In the context of climate change, the observed increase in average surface temperatures causes an acceleration of plant phenology progression, exposing vulnerable green bud structures to a higher risk of late frost damage (Alikadic et al., 2019; Masson-Delmotte et al., 2021). In the context of some extreme weather and climate events have increased in frequency and intensity due to global warming, adaptive techniques in viticulture have become necessary to mitigate the resulting negative impacts (Ferrara et al., 2022).

The adaptation and resistance of the grapevine to low temperatures during the dormancy period involve the realization of processes of accumulation and dynamics of biochemical compounds in tissues, with direct implications for protecting them against the destructive action of intracellular freezing (Holzapfel et al., 2010; Mohamed et al., 2012; Babajamali et al., 2022; Monteiro et al., 2022). The carbohydrate content of grapevine buds undergoes significant changes in an annual cycle, and there is a continuous interdependence between the content of soluble sugars and starch (Rogiers et al., 2011; Tixier et al., 2019). An important role is played by starch, which, through the hydrolysis process starting in January, leads to the formation of soluble sugars with a cellular protective function due to the promotion of water retention through osmotic means, thereby lowering the cryoscopic point of cellular sap (Zapata et al., 2004; Lebon et al., 2008). Several studies indicate that during winter, grapevine roots contain rich reserves of starch and sugars, with starch being present in all tissues of the bark and wood (annual or multiannual), actively contributing to the dynamics of carbohydrates in grapevine shoots (Caprara & Pezzi, 2013; Liu and Sherif, 2019; Călugăr et al., 2022; Ferrara et al., 2022; Costea et al., 2023). The larger the reserves of carbohydrates accumulated in the wood, the better the wood maturation, and the plant becomes more resistant to low temperatures during the dormancy period (Cichi et al., 2016; Călugăr et al., 2019; Horiuchi et al., 2021). Essential climate characteristics in a vineyard area have a decisive impact on the evolution of the biochemical, biological, and physiological processes of grapevines during the dormancy period. These characteristics include the average minimum temperature, absolute minimum temperature, and others (Grant et al., 2013; Onache et al., 2020; Costea et al., 2021; Hernandez et al., 2021; Căpruciu et al., 2023). Among these, temperature is a crucial factor that affects nearly all aspects of plant growth and development, with grapevines (*Vitis* spp.) being quite sensitive to extreme temperatures (Bernardo et al., 2018; Venios et al., 2020; Cichi et al., 2021).

Pedo-climatic factors, as well as orographic characteristics and the grapevine variety itself, directly influence the solubility and transport of synthesized organic substances (carbohydrates, amino acids, etc.), ensuring a continuous regulation of metabolic processes involving synthesis and hydrolysis. This regulation has an impact on enhancing resistance to freezing in grapevine wood (Field et al., 2009; Bucur and Dejeu, 2020; Costea and Căpruciu 2022; Cichi et al., 2023; Costea et al., 2023).

The present study is focussed on the assessment of the content and dynamics of the starch and soluble sugars during the dormancy season of some wine grape cultivars grown in two wine-growing area from Oltenia and Muntenia Hills Region.

MATERIALS AND METHODS

Sampling Sites. The Banu Mărăcine viticultural center is located at an altitude of 176 m, with 44º19' north latitude and 23º48' east longitude. Banu Mărăcine belongs to the A_3 oenoclimatic zone, which includes viticultural centers producing mainly high-class red and aromatic wines, and, secondarily, quality table wines (Teodorescu et al., 1987). Geographically, the Șimnicu de Sus area is located in the South-West of Romania, at 44°24'23 "N, 23°48'09" E, and is characterized by mild winters and long summers, with high sunshine duration and low rainfall (Table 1), climatic conditions required for the synthesis of carbohydrates. In the Banu Mărăcine and Șimnicu de Sus wine areas, the climate is temperate-continental with Mediterranean influence, characterized by mild winters and hot summers with a high number of tropical days, where maximum temperatures often exceed 30˚C. The experiments were conducted within the Banu Mărăcine viticultural area in parcels situated in identical orographic conditions (plateau-type area with slightly levigated reddish-brown soil) and within Șimnicu de Sus wine-growing area, characterized by slope-type area with medium-eroded reddish-brown soil.

Table 1. Description of the sampling sites for black grapes

Sampling sites	Șimnicu de Sus	Banu Mărăcine
Altitude (m)	175	176
Average January temperature (°C)	+1.12°C	+2.31°C
Radiation	Hight	Hight
Precipitation (mm)	565	540
Soil	slope-type area, medium eroded reddish-brown soil	plateau-type area, slightly leached reddish-brown soil

Biological material

The cultivars included in the study are grafted on Berlandieri x Riparia SO4 rootstock, planted with a spacing of 2 x 1.2 m, exhibiting semi-high growth with a multiple Guyot cutting system, and a crop load of 40 buds/vine. These have been: 'Cabernet Sauvignon', 'Fetească neagră' and 'Merlot'.

The observations and determinations were conducted for the 'Cabernet Sauvignon', 'Fetească neagră', and 'Merlot' wine grape varieties during the 2022-2023 period within Banu Mărăcine - the Didactic Research Station of the University of Craiova and Șimnicu de Sus wine-growing area.

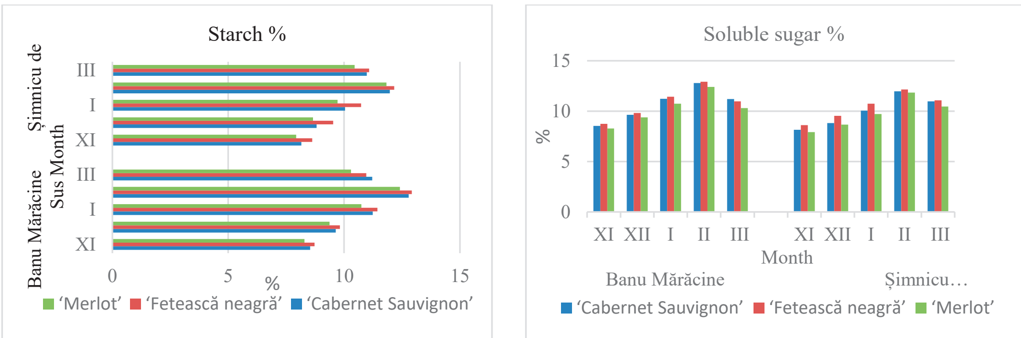
Parameters determined were done on both annual and multiannual wood (3 years), collected at a 10-day interval during the dormant period (XI-III). This involved observing the dynamics of the main biochemical compounds (soluble sugars % and starch %) on 10 canes/variety/type of wood harvested from 10 vines of each variety. The analyses were carried out at the Research Center for Life Sciences and Biotechnologies of the Faculty of Horticulture, University of Craiova.

Methods. The monitoring of the minimum temperature during the dormant period was conducted at the Banu Mărăcine Meteorological Station, and for Șimnicu de Sus, the AccuWeather application was utilized. Carbohydrates were determined through spectrophotometric analysis. In this method, soluble sugars were extracted using an 80% volume alcohol solution, the starch with a 52% volume perchloric acid solution, followed by treatment with a 0.2% anthrone ($C_{14}H_{100}$) solution. The obtained colour intensity (with transparent blue-green colour shades) was measured colorimetrically using a UV-VIS Spectrophotometer at a wavelength of 620 nm (Călugăr et al., 2019). The data resulting from the spectrophotometric reading were calculated as follows: Sugars (%) = E_c-E_a/E_b x 50; Starch (%) = E_d-E_a/E_b x 50; where E_a, E_b, E_c, E_d=extractions of solutions a,b,c,d; 50 = concentration of the standard solution.

Statistical Analysis. Each variable was examined using analysis of variance (ANOVA). The differences between the mean values of biochemical compounds were tested with Tukey's HSD test (using the SPSS 16 program), and the results were expressed as mean ± standard deviation (SD). Additionally, the coefficient of variation (CV %) was calculated.

RESULTS AND DISCUSSIONS

The adaptation and resistance of the vine to low temperatures (absolute minimum temperature) during the period of vegetative rest involves the realization of processes of accumulation and dynamics of some biochemical compounds in the tissues. In both studied viticultural areas, it has been determined that until the end of January, there was a constant decrease in starch content. During the month of February, the recorded decreases were noticeable, influenced by the recorded minimum temperatures, and starting from March, a slight increase in starch content became evident, particularly in the annual wood of all analysed cultivars (5.17% in annual wood compared to 4.28% in multiannual wood for the 'Cabernet Sauvignon' in the Banu Mărăcine viticultural area). The dynamics of starch (%) and soluble sugars (%) content in the annual vine wood during the dormant season in Banu Mărăcine and Șimnicu de Sus is presented in Figure 1 (a and b). Analysing the dynamic content of carbohydrates in the annual canes of the studied cultivars, it is observed that 'Fetească neagră' records the highest values of soluble sugars content (13.11% on 22.02.2023 in Banu Mărăcine and 12.93% on 22.02.2023 in Șimnicu de Sus), as shown in Figure 1b. Against the backdrop of absolute minimum temperatures in February (-12.6ºC), it is noted that all varieties registered maximum quantities of soluble sugars (13.11% for 'Fetească neagră', 13.06% for 'Cabernet Sauvignon', and 12.87% for 'Merlot' in the Banu Mărăcine viticultural area). In the Șimnicu de Sus viticultural area, maximum values of soluble sugars content were also recorded in February, albeit lower compared to those obtained for the same cultivars cultivated in the Banu Mărăcine viticultural area (12.93% for 'Fetească neagră', 12.75% for 'Cabernet Sauvignon', and 12.55% for 'Merlot'), as illustrated in Figure 1b. Alongside the decrease in starch content in all studied cultivars, there is a slow and continuous increase in the content of soluble sugars. This increase became evident from the second decade of January until the end of February, amid absolute minimum temperatures of -12.6ºC in Banu Marăcine and -11.2ºC in Șimnicu de Sus. Starting from the first decade of March, both annual and multiannual cane starch values began to rise, while the content of soluble sugars followed a decreasing dynamic with variations between different cultivars based on their metabolic characteristics (Figure 1a and 1b). Also, Călugăr et al. (2019) state that due to climatic conditions during dormancy, starch began to resynthesize already in January and February, with significantly high levels for all cultivars. Field et al. (2009) and Zufferey et al. (2012) note that the conversion of starch into soluble carbohydrates occurred during the winter, coinciding with lower temperatures. These results confirm earlier observations made on different grapevine cultivars and other woody species. Towards the end of the dormancy period (March 24, 2023), 'Fetească neagră' recorded the highest content of soluble sugars, both in annual wood (9.94% in Banu Mărăcine and 9.82% in Șimnicu de Sus) and in multiannual wood (9.43% in Banu Mărăcine and 8.71% in Șimnicu de Sus). The lowest content of soluble sugars at the end of the dormancy period was noted for the 'Merlot' (Figure 1b). The quality of 'Fetească neagră' and ‘Merlot’, depending on the cultivation area and climatic conditions, was also studied by Călugăr et al. (2019), Onache et al. (2020), as well as Bucur and Dejeu, (2020). The impact of climate change on grapevine phenology, investigating the role of cultivars and microclimates in the areas, was studied by Alikadic et al. (2019). The average content of soluble sugars (%) during the dormancy period 2022-2023 in annual woody shoots ranged from 9.81% (‘Merlot’ in Șimnicu de Sus) to 11.02% (‘Fetească neagră’, Șimnicu de Sus). In the Șimnicu de Sus viticultural area, there were no statistically significant differences between culttivars regarding the content of soluble sugars in annual woody shoots (Table 2). Statistically significant differences between ‘Merlot’ and ‘Fetească neagră’ ($p \leq .05$) and ‘Cabernet Sauvignon’ ($p \leq .05$) were only observed in the Banu Mărăcine viticultural area. Furthermore, regarding the influence of the viticultural area on the average content of soluble sugars (%) in annual woody shoots for each of the three studied cultivars, no statistically significant differences were established. The average content f soluble sugars (%) during the dormancy period in multiannual wood ranged from 8.76% (‘Merlot’, in Șimnicu de Sus) to 10.03% (‘Fetească neagră’, Banu Mărăcine). Statistically significant negative differences in the content of soluble sugars in multiannual wood were observed between ‘Merlot’ and ‘Fetească neagră’ ($p \leq .05$) in the Șimnicu de Sus viticultural area. Additionally, statistically significant positive differences in the average content of soluble sugars (%) during the dormancy period in multiannual wood were noted for ‘Cabernet Sauvignon’ ($p \leq .05$) and ‘Merlot’ ($p \leq .01$) in the Banu Mărăcine viticultural area compared to the Șimnicu de Sus area. The starch content (%) during the dormancy period 2022-2023 in annual woody shoots ranged from 4.84% (‘Merlot’, in Șimnicu de Sus) to 6.07% (‘Fetească neagră’, Șimnicu de Sus).



(a) (b)

Figure 1. The dynamics of the content of starch (a) and soluble sugars (b) in annual vine wood during the dormancy period in Banu Mărăcine and Șimnicu de Sus (XI- November, XII-December; I-January, II- February; III-March)

Table 2. The content of the carbohydrate during the dormant season*

Cultivar	Wine Area	Soluble sugars %				Starch %			
		Annual woody shoot	CV%	Multiannual wood	CV%	Annual woody shoot	CV%	Multiannual wood	CV%
‘Cabernet Sauvignon’	Banu Marăcine	10.91 ± 0.21^{aA}	1.92	9.85 ± 0.23^{aA}	2.33	5.73 ± 0.47^{aA}	8.20	5.55 ± 1.01^{aA}	18.19
	Șimnicu de Sus	10.60 ± 0.82^{aA}	7.73	9.06 ± 0.40^{abB}	4.42	5.64 ± 0.44^{aA}	7.80	5.37 ± 0.73^{aA}	13.59
‘Feteasca neagra’	Banu Marăcine	10.81 ± 0.10^{aA}	0.92	10.03 ± 0.24^{aA}	2.39	5.48 ± 0.68^{aA}	12.41	5.88 ± 0.67^{aA}	11.39
	Șimnicu de Sus	11.02 ± 0.67^{aA}	6.08	9.82 ± 0.40^{aA}	4.07	6.07 ± 0.96^{aA}	15.82	5.66 ± 0.77^{aA}	13.60
‘Merlot’	Banu Marăcine	10.23 ± 0.27^{bA}	2.64	9.55 ± 0.13^{aA}	1.36	4.84 ± 0.59^{aA}	12.19	4.62 ± 0.71^{aA}	15.37
	Șimnicu de Sus	9.81 ± 0.41^{aA}	4.18	8.76 ± 0.25^{bB}	2.85	5.10 ± 0.69^{aA}	13.53	4.77 ± 0.68^{aA}	14.26

* Note: Data are Means±SD; Means separation by HSD Tukey`s test at $p\leq0.05$. Means with the same superscript are not statistically significant difference. In the column, lowercase letters indicate the significance of differences between cultivars for each studied viticultural area, while uppercase letters represent the significance of differences between the two areas for each cultivar.

In multiannual wood, the starch content (%) during the dormancy recorded values between 4.62% ('Merlot', in Banu Măracine) and 5.88% ('Fetească neagră', in Banu Măracine). Although there were some differences in starch content (%) in annual woody shoots and multiannual wood among the studied cultivars and between the two areas, these differences were not statistically significant (Table 2).

CONCLUSIONS

The dynamics of the starch and soluble sugars content show variations depending on the cultivar and thermal conditions from November to March, with the most intense metabolic transformation of starch into soluble sugars being recorded in the months of January and February.

Regarding the carbohydrate content during the dormancy, significant differences between the two viticultural areas were highlighted only concerning the content of soluble sugars (%) in multiannual wood for the 'Merlot' and 'Cabernet Sauvignon'.

In the Banu Mărăcine viticultural area, the average content of soluble sugars (%) in multiannual wood for both cultivars was higher compared to the Șimnicu de Sus area.

Significantly negative differences between cultivars regarding the content of soluble sugars (%) in annual woody shoots were determined for the 'Merlot' compared to 'Cabernet Sauvignon' and 'Fetească neagră' in Banu Mărăcine. Significantly negative differences between varieties regarding the content of soluble sugars (%) in multiannual wood were noted between 'Merlot' and 'Fetească neagră' in the Șimnicu de Sus viticultural area.

As for the starch content (%) in annual woody shoots and in multiannual wood, no statistically significant differences were observed either between the studied cultivars or between the two areas.

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EVALUATION OF THE POLYPEHNOL EXTRACTS FROM VINE CANES (*Vitis vinifera* L.) OBTAINED BY AN IMPROVED EXTRACTION METHOD

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Abstract

Waste vegetal materials generated during vine pruning are rich in bioactive molecules, especially polyphenols, therefore their valorization received considerable attention in the last years. This work focuses on evaluating the polyphenol composition and antimicrobial activity of vine cane extracts obtained with an improved method using a pre-treatment with cell wall degrading enzymes and fluidized bed extraction. Different quantities of canes (1 to 20 g) were used to obtain several extracts. Total phenolic content (TPC), hydrolysable and condensed tannins, antimicrobial and antioxidant activity (DPPH test) were determined. The extract obtained from 5 g of dry vine cane showed the highest antioxidant activity (85.64±0.22%), a high value of TCP (83.85±4.62 mg GAE/g dw) and a 2.44 times higher antimicrobial activity compared to antibiotic ciprofloxacin on E. coli ATCC 8739. However, the highest gallotannin content (16.26±0.03 mg tannin acid/g dw) was detected in the extract obtained from 20 g of vine canes, while the condensed tannin was low in all samples.

Key words: *improved extraction method, grapevine cane, polyphenol composition, antioxidant activity, antimicrobials.*

INTRODUCTION

Cultivating vines for grape and wine production is a longstanding and significant agricultural pursuit on a global scale. However, the wine-making industry produces several waste materials, such as grapevine stems and leaves, and by-products from the vinification process, including grape pomace, wine marc and wine lees (Ferrer-Gallego et al., 2022; Taladrid et al., 2023). It is considered that annually 2 to 5 tonnes/hectare of grapevine canes are produced (Arvanitoyannis et al., 2006). Although the vine canes represent 25% of total winery wastes, only in the last years their valorization has received considerable attention, as they are rich in bioactive compounds, especially dietary fibers and polyphenols (Baroi et al., 2022; Rodrigues et al., 2023). It contains a rich array of polyphenols, including stilbenes, gallotannins, proanthocyanidins and phenolic acids (Aliaño-González et al., 2020; Escobar-Avello et al., 2019; Escobar-Avello et al., 2021). The variation in concentration of polyphenolic compounds is correlated with the grape variety, the cultivation conditions, harvesting time and storage conditions (Gorena et al., 2014; Piñeiro et al., 2017). Thus, Escobar-Avello et al., 2019 using high-resolution mass spectrometry identified in grape cane extract a total of 75 phenolic compounds, the most abundant being proanthocyanidins and stilbenoids and their oligomers (Escobar-Avello et al., 2019). In a recent review on the grapevine chemical composition of different vegetative parts, Goufo et al., 2020 found that flavonols (83.43% of total phenolic levels) and flavan-3-ols (61.63%) are the main compounds in stems and leaves (Goufo et al., 2020). The richest in stilbene were Pinot noir and Gewurztraminer cane extracts, containing up to 5800 mg/kg dry weight (Aliaño-González et al., 2020). These phytochemicals, especially stilbenes, exhibit potent antioxidant effects, scavenging free radicals and combating oxidative stress (Aliaño-González et al., 2020). Additionally, grape vine cane extract is replete with vitamins, minerals, and amino acids, further enhancing its nutritional profile. Therefore, the antioxidant, anti-inflammatory, and anti-microbial properties of vine cane extracts have been tested as potential treatments for cardiovascular and neurological diseases, and even cancer, with promising results (Dani, 2010; Selma et al., 2021; Quero et al., 2021; Empl et al., 2018). There is a growing demand to find natural compound mixtures that can be used in the development of innovative products for the nutraceutical industry (Dinu et al., 2023). Different methods for extracting polyphenols from grapevine canes have been studied and recently reviewed (Baroi et al., 2022; Aliaño-González et al., 2020). In this process, the efficiency of extraction methods to recover bioactive compounds with health-promoting effects is very important. Enzyme-based extraction method has proved to increase the amount of total (free and polysaccharides bound) phenolics and antioxidant activity of extracts (Hong et al., 2013).

Therefore, this study aims to evaluate the efficacy of an improved method to extract polyphenols from vine canes. This is based on a cane cuts pre-treatment with plant cell wall degradation enzymes, to help increase the extraction of total phenolics.

MATERIALS AND METHODS

Samples collection and preparation. Vine canes from red (Cabernet Sauvignon) and white (Tămâioasă Românească) grape varieties were obtained in the spring of 2023, from Pietroasa winegrowing center (Romania). The samples were cut into 2-5 cm sections and oven-dried at 50^0C, 24h, and then milled. Samples containing 1 g, 5 g, 10 g, and 20 g of the substrate were coded P1-P4, respectively

Pre-treatment procedure. Samples with different substrate concentrations (P1-P4) were mixed with water and 0.5% Viscozyme L (Sigma-Aldrich), a cell wall degrading enzymes complex, for 24 h, 200 rpm.

Extraction methods. Fluidized bed extraction with IKA-RET 135.2 was used in the presence of a mix of ethanol: water (1:1 v/v). The pH of the extracts was 5.5-6.

Chemical analysis. The total phenolic content (TPC) was assessed using ISO 14502-1; 2005 and expressed as gallic acid (GAE) eq. Condensed tannins/proanthocyanidins (calculated as cyanidin chloride eq.) were determined with a modified butanol-HCl method (Scalbert et al., 1989; Vermerris and Nicholson, 2006) and hydrolysable tannins/gallotannins (calculated as tannic acid eq.) using KIO_3 method (Haslam *et al.*, 1965; Bate-Smith, 1977; Willis and Allen, 1998).

Antioxidant and antibacterial activities. The DPPH radical scavenging activity (%) was determined based on a protocol previously reported, with ascorbic acid as standard (AcS 1%) (Vamanu and Nita, 2013). Disk-diffusion method was used to test the antimicrobial effect of non-alcoholic extracts on *Escherichia coli* ATCC 8739. The results were compared to the susceptibility of the antibiotic ciprofloxacin CIP1 (Oxoid), which presents an inhibition zone of 9±1.41 mm. Antimicrobial activity ratio was calculated as extract inhibition zone/CIP1 inhibition zone.

Statistical analysis. The results presented in figures are average values of at least three replications. Standard deviation was also calculated with the software package Excel.

RESULTS AND DISCUSSIONS

Grapevine cane extract is a treasure trove of bioactive compounds (Souquet et al., 2000). Therefore, in recent years, the valorization of vine grape extract has attracted significant attention for its potential contributions to health and wellness (Niculescu and Ionete, 2023).

This work focused on evaluating the polyphenol composition, antioxidant and antimicrobial activity of vine cane extracts obtained with an improved method. The samples P1-P4 with different substrate concentrations (1 g, 5 g, 10 g, and 20 g, respectively) were pre-treated for 24 h with 0.5% solution of cell wall degrading enzymes, followed by fluidized bed extraction with a mix of ethanol: water (1:1 v/v). The polyphenol composition of different extracts are shown in Figures 1-2.

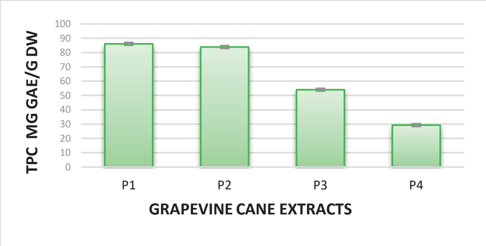

Figure 1. The effect of substrate concentration (samples P1-P4 containing 1 g, 5 g, 10 g, and 20 g of substrate, respectively) on total phenolic content (TPC) of grapevine cane extracts

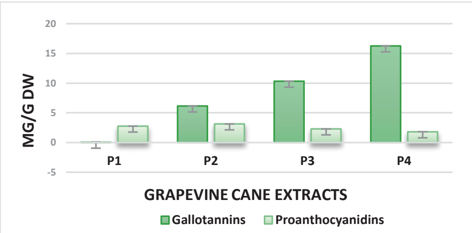

Figure 2. The effect of substrate concentration (samples P1-P4 containing 1g, 5 g, 10 g, and 20 g of substrate, respectively) on hydrolysable and condensed tannins content of grapevine cane extracts

The extract obtained from 5 g of vine cane (P2) showed a high value for TPC (83.85±4.62 mg GAE/g dw), while the gallotannin content was 6.15±0.01 mg/g dw. However, the sample (P4) obtained after bed fluidized extraction of 20 g substrate showed low TCP, but increased gallotannins content (16.26±0.03 mg/g dw). The total phenol and gallotannin contents of grapevine cane extracts were influenced by the amount of substrate used, but not necessarily in a direct dependence with the quantity. Thus, higher TCP was detected in plant extracts obtained from up to 5 g grapevine canes. Hydrolysable tannin content gradually increased with substrate concentration, while the condensed tannins were low in all samples, with a maximum value of 3.13±0.001 mg/g dw for sample P2. Dorosh et al., 2022 using an optimized procedure of subcritical-water extraction obtained cane extracts with TPC 181±12 mg GAE/g dw from one of the Portuguese vine varieties (Dorosh et al., 2020). However, the gallic acid content of up to 15 mg GA/g dw was detected (Dorosh et al., 2020). In another study, methanol extracts obtained by ultrasound-assisted extraction from three Greek vine varieties (Mavrodaphne, Muscat and Rhoditis) were compared. The higher total phenolic content was 374.76 mg GAE/g dw for extract from the Mavrodaphne variety and the predominant phenolics were gallic acid, caffeic acids, quercetin and quercitrin (Veskoukis et al., 2020). It was thus suggested that methods based on plant cell wall degradation increased the polyphenolic content. These works also found that higher levels of total phenols and the presence of gallic acid increased the antioxidant activity of the extracts (Dorosh et al., 2020; Veskoukis et al., 2020).

Three of four grapevine cane extracts had a high antioxidant activity detected using DPPH test (Figure 3). Sample P2 showed the highest antioxidant activity (85.64±0.22% inhibition), probably correlated with the TCP content. The antioxidant activity for samples obtained from 10 g (P3) and 20 g (P4) grapevine canes was slowly lower than that for P2 sample. However, using the lowest amount of canes (1 g) significantly decreased the antioxidant activity to 26.46±1.10% inhibition.

Grape canes of black and white *Vitis vinifera* grapes varieties were collected from Czech vineyards and phenols extracted were by 40% ethanol (Gharwalova et al., 2020). The antioxidant activity of the samples (DPPH test) ranged between 29.46-71.46% inhibition and the TPC varied between 6.30-20.44 mg GAE/g dw (Gharwalova et al., 2020). Testing the antioxidant activities in polyphenol-rich grape cane extracts from 44 European varieties, Ferrier et al., 2022 noted that extracts from Savagnin blanc, Villard noir and Magdeleine noire des Charentes had higher antioxidant capacities based on ORAC, ABTS, DPPH, FRAP, CUPRAC and chelation assays (Ferrier et al., 2022). Moreover, the higher level of some molecules, especially E-resveratrol (3), E-piceatannol (4), E-ε-viniferin (13), hopeaphenol (36), isohopeaphenol (37) and Z/E-vitisin B (41) were found to be the main drivers of ABTS and DPPH capacities.

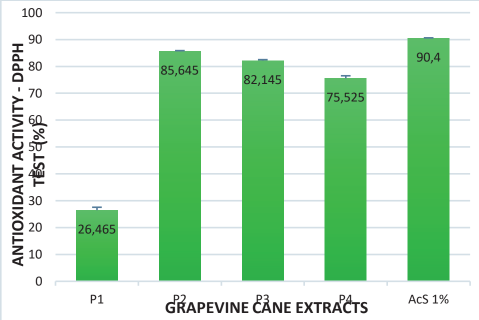

Figure 3. Antioxidant activity of grapevine cane extracts

All extracts obtained after enzyme pre-treatment and bed fluidized extraction proved to have a strong antibacterial activity on strain *E. coli* ATCC 8739 (Figure 4). In the case of P2 and P4 samples, the antimicrobial activity ratio was 2.44 higher compared to the antibiotic ciprofloxacin (CIP1). However, the larger inhibition zone (25±1.42 mm) was noted for extract obtained from 10 g of vine canes (P3).
In another work *Vitis vinifera* var. Red Globe cane extracts were used as a sanitizer and were effective in reducing the populations of pathogens *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella enterica* subsp. *enterica* serovar *typhimurium*, and *Escherichia coli* O157: H7 (Vázquez-Armenta et al., 2017). Some promising results were obtained by exploiting the antioxidant and/or antimicrobial activity of vine canes or their extracts. They were able to replace the most common oenological additives and inhibit the activity of major food pathogens (Troilo et al., 2021; Blackford et al., 2021).

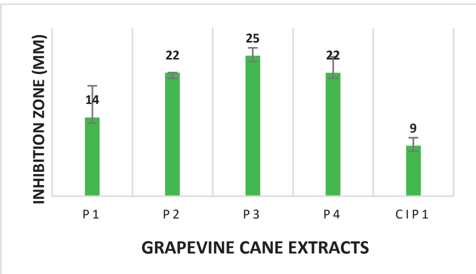

Figure 4. Antimicrobial activity of grapevine cane extracts and antibiotic ciprofloxacin (CIP1)

CONCLUSIONS

The grapevine stems are the least valorized subproduct from the wine industry despite being produced in huge amounts. The work proposes a new approach to extract polyphenols from vine canes based on enzyme-assisted pre-treatment and fluidized bed extraction which is a relatively simple and cost-effective method. A high value for total phenolic content (83.85±4.62 mg GAE/g dw), and increased gallotannins composition were obtained after extraction from 5 g of substrate (P2). As regards the antioxidant activity, the DPPH value for this extract was the highest (85.64±0.22%), while it showed strong antimicrobial activity on the pathogen *E. coli*.
This approach is environmentally friendly, while the polyphenolic profile of obtained vine cane extracts proves their potential to be exploited by the nutraceutical industry. Further works will investigate the polyphenol-rich cane extract modulation effects on dysbiotic gut microbiota and their health-promoting effects.

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ROMANIAN VINEYARD AREAS AND THE EVOLUTION OF WINES WITH TRACEABILITY IN THE PERIOD 2007-2022, USING EUROPEAN RESTRUCTURING/RECONVERSION FUNDS

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Abstract

This study provides an overview of the substantial changes in the structure of the wine grape assortment in Romania through the use of European funds allocated for vineyards reconversion/restructuring. Between 2007 and 2022, both new international varieties, which previously existed only in ampelographic collections, and old Romanian varieties, which are more adaptable to climate change and are increasingly appreciated by the wine drinking public, were introduced into the Romanian wine assortment. The study presents an update on the evolution of the areas cultivated with the most widespread wine grape varieties destinated for wines with protected designation of origin, protected geographical indication and varietally. On this basis, the quality level of the wines obtained during the 15 years studied was analyzed, based on the assessments made by the specialist tasting committees of the governmental Body which, on the basis of the traceability ensured by the producer, guarantees their origin and authenticity.

Key words*: assortment, PDO, PGI, tasting, winegrapes.*

INTRODUCTION

With Romania's accession to the European Union, structural funds have been accessed to the same extent as in the other Member States, including funds that have enabled the restructuring and/or reconversion of vineyards in order to add value to the Romanian wine sector and bring it into harmony with the European common market.
The specific Romanian legislation has been revised, updated and supplemented, through the provision that allows the annual modification of the support programs for the following year, so that they can be adjusted to take into account any new condition that was not foreseen at the time of their initial presentation (Reg. 555/ 2008).
The support for measures to reconversion/ restructuring of vineyards was provided from the state budget through the budget of the Ministry of Agriculture and Rural Development and from the European Union's non-repayable external contribution from the European Agricultural Guarantee Fund (Hot. 578/2014).
The beneficiaries of the financial support were producers in the wine sector, private individuals or legal entities operating in the wine sector and their associative forms (Hot. 868/2018).
Reconversion/ restructuring programmes are all eligible measures which, by their implementtation, lead to an increase in the competitiveness of wine producers by adapting production to market demand. (Ord. 247/2008)
Varietal reconversion consists of changing the existing wine grape varieties in the vineyard to qualitatively superior varieties or clones of these varieties in order to adapt production to market requirements (Ord. 247/2008).

Restructuring is the relocation of parcels and consists of planting vineyard parcels located in unfavourable conditions on other sites offering favourable climatic and soil conditions on the same area, or of modernising the vineyard site, through a set of eligible measures leading to increased efficiency and improved quality of wine products (Ord. 247/2008).

In 2014, the specific legislation was supplemented by replanting following compulsory vineyards deforesting for sanitary or phytosanitary reasons by exchanging existing varieties in the crop that have been affected by diseases with the same varieties or with other varieties or clones of these varieties in order to adapt production to market demand. Romania's National Support Program for 2009-2013 reported the total absorption of structural funds for viticulture (Ord. 1763/2014).

Initially legislated until 2019, the support measures for the reconstruction of vineyards in Romania have been extended until 2023 by a new ministerial order (Ord 1508/2018).

The content of any support program is regulated at European level (Reg. 1150/2016). All plans for the reconversion/ restructuring of the Romanian vineyards, carried out using national and European support measures, have benefited from cross-compliance with the specific environmental and climate objectives (Reg. 2289/2021).

This study will show that as a previous research study predicted “from 2013-2016, the planting rate started to decrease” (Antoce & Călugăru, 2017).

Since 2007, immediately after Romania's ace-ssion to the European Union, the reconversion/ restructuring of vineyards began, which was also legislated, following the European model.

MATERIALS AND METHODS

In order to characterize the evolution of the area of vineyards in Romania in the period 2007-2022, we have chosen from the list of varieties allowed for the reconversion/ restructuring of vineyards, according to Romanian and European legislation, the grapevine varieties that have had a remarkable evolution: increasing, decreasing or, surprisingly, after a steep upward or downward trend, remained constantly.

The European regulation on the common market policy for the wine sector entered into force on 1 August 2008, so in this study 2007 as chosen as a starting point for comparison. (Reg. 1237/2007).

The period analysed in this study was 2007-2022. All the vineyard areas in this study were authorised by the National Office of Vine and Wine Products, in each year of those studied, for the production of wines with a controlled designation of origin, geographical indication or variety denomination, ensuring their traceability.

The data, collected from the National Office of Vine and Wine Products, from the digital database called National Register for Vine Plantations, have been statistically processed and interpreted.

RESULTS AND DISCUSSIONS

The reconversion/ restructuring programmes for vineyards that have not reached the end of their production cycle have also brought major changes for Romania, both in the wine assortment and in the more judicious location of vineyards, with their migration from the lowlands to the hills where possible. Between 2007-2022 with the support of European funds, the area of Romanian vineyards increased from 78812.9 ha to 83183.8 ha, in an up-and-down manner (Figure 1).

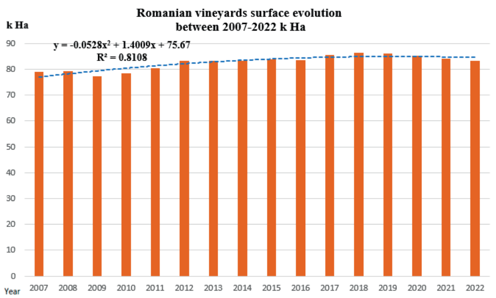

Figure 1. Vineyards surface evolution 2007-2022 (kHa)

Most of the areas planted to certain international wine grape varieties have decreased over the 16 years analysed in this study (2007-2022). ’Aligoté’ had a very significant decrease (R^2 = 0.9344) from 7727.55 to 5093 ha (Figure 2).

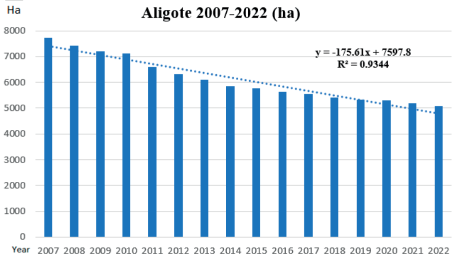

Figure 2. ‘Aligoté’ surface decline 2007-2022

’Sangiovese’ decreased significantly from 105.59 to 75.69 ha (R^2 = 0.8249) (Figure 3).

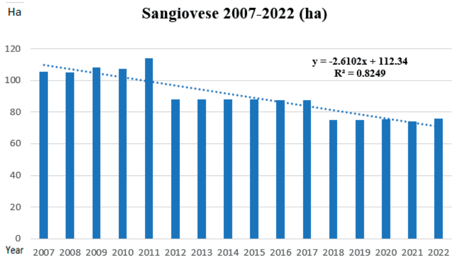

Figure 3. ‘Sangiovese’ surface decline 2007-2022

’Portugais bleu’ decreased significantly from 409.96 to 28.87 ha (R^2 = 0.8103) (Figure 4).

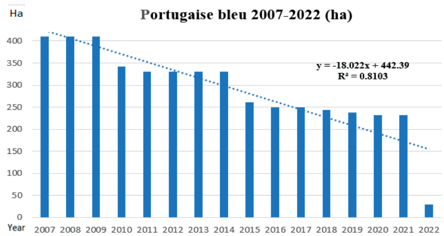

Figure 4. ‘Portugais bleu’ surface decline 2007-2022

’Zweigelt’ decreased after 4 years after the reconversion start, significantly (R^2 = 0.7739), from 109.95 to 75.48 ha) (Figure 5).

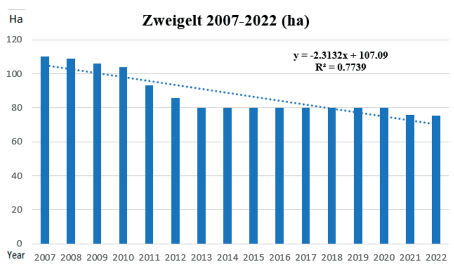

Figure 5. ‘Zweigelt’ surface decline 2007-2022

’Riesling italian’ had a significant decrease (R^2 = 0.7569), from 8354.87 to 6861 ha (Figure 6).

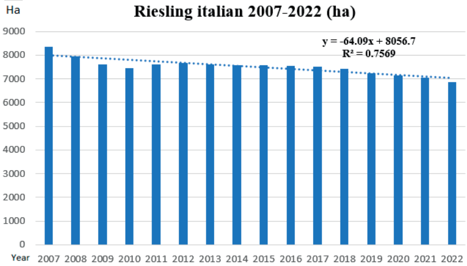

Figure 6. ‘Riesling italian’ surface decline 2007-2022

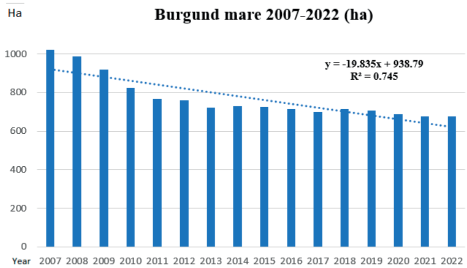

Figure 7. ‘Burgund mare’ surface decline 2007-2022

’Burgund mare’, from 1020.09 to 676.3 ha, had a significant decrease too (R^2 = 0.745) (Figure 7). ’Merlot’ decreased from 11472.65 to 10973 ha) having very large and frequent fluctuations (R^2 = 0.5321) (Figure 8).

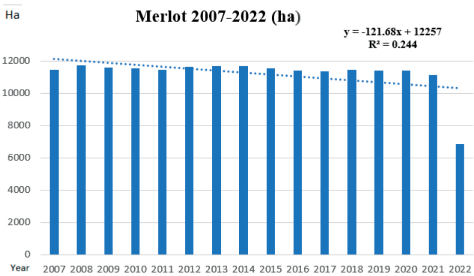

Figure 8. ‘Merlot’ surface decline 2007-2022

Other wine grape varieties or clones have replaced all of these decreased varieties.
The same downward trend is observed in the indigenous varieties. ’Feteasca regala’ has a very significant decrease (R^2 = 0.8783) from 13949.88 to 12012 ha (Figure 9).

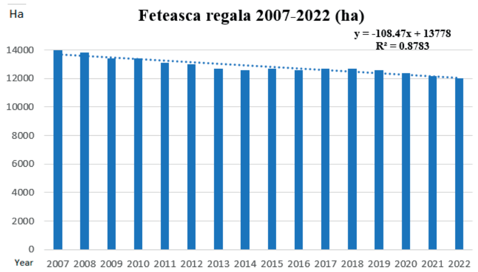

Figure 9. 'Feteasca regala' surface decline 2007-2022

'Majarca' decreased from 68.99 to 7.78 ha, with a significant decrease as seen in Figure 10, and $R^2 = 0.7762$.

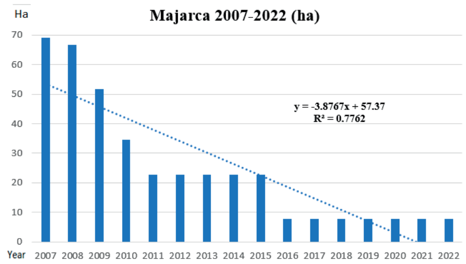

Figure 10. 'Majarca' surface decline 2007-2022

'Babeasca neagra' decreased from 3545.46 to 2504.5 ha (Figure 11). The data show a decrease in interest in this variety from producers, probably also due to the competition it has from producers from the Republic of Moldova.

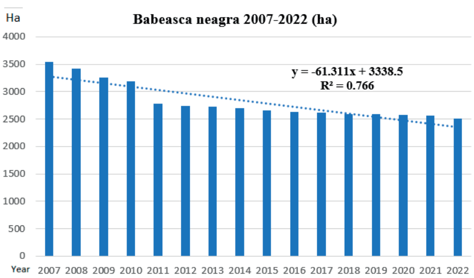

Figure 11. 'Babeasca neagra' surface 2007-2022

'Feteasca alba' decreased from 15075.34 to 11872.6 ha (Figure 12) with a $R^2 = 0.7492$. The decrease of this variety could be due to the low resistence to the disease and the need of many phytosanitary treatments. Although, is a autochthonous variety and which is a different product for international wine market.

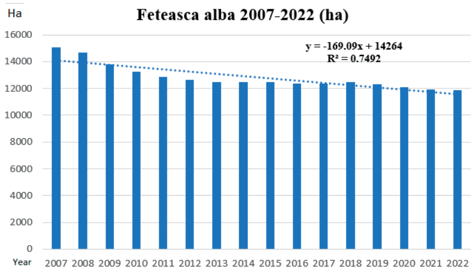

Figure 12. 'Feteasca alba' surface decline 2007-2022

'Aromat de Iasi' decreased significantly from 65.87 to 54.31 ha (Figure 13).

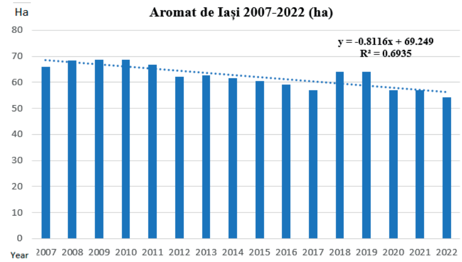

Figure 13. 'Aromat de Iasi' surface decline 2007-2022

'Mustoasa de Maderat' decreased from 421.5 to 284.03 ha (Figure 14).

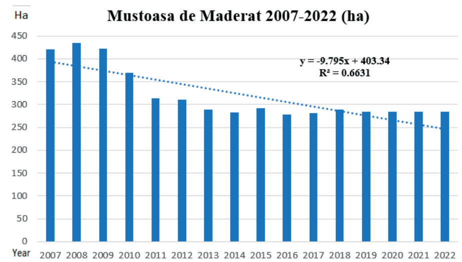

Figure 14. 'Mustoasa de Maderat' surface 2007-2022

'Iordana' (from 433.97 to 297.4 ha) (Figure 16).

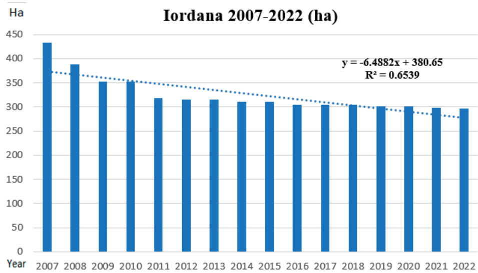

Figure 15. 'Iordana' surface decline 2000-2022

’Cramposie’ decreased from 660.74 to 358.09 ha (Figure 16).

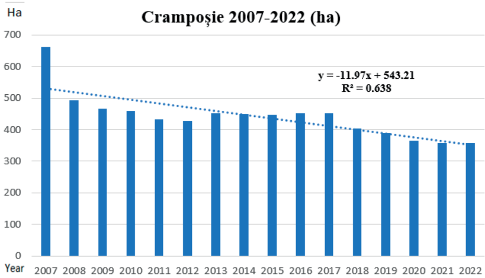

Figure 16. ‘Cramposie’ surface decline 2007-2022

’Babeasca gri’ decreased with one abrupt fluctuation, from 332.49 to 295.06 ha (Figure 17).

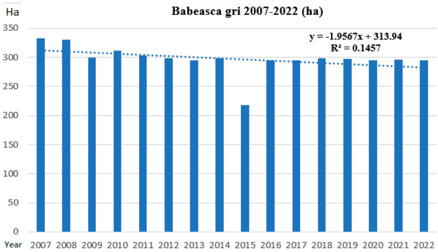

Figure 17. ‘Babeasca gri’ surface decline 2007-2022

In the case of other varieties, either a decrease, followed by stagnation, or an increase, followed by constant maintenance of the plantation size, is observed over the 16 years analysed (2007-2022).

’Muscat Ottonel’ increased very significant (R^2 = 0.9409) in the first 10 years after the start of the Reconversion and then remain on a plateau, from 337.07 to 4944 (Figure 18).

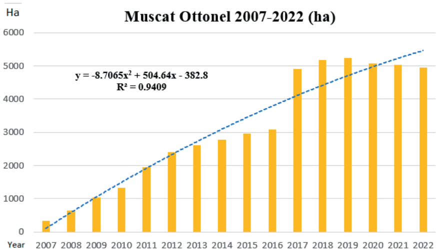

Figure 18. ‘Muscat Ottonel’ surface 2007-2022

’Chardonnay’ increased significantly (R^2 = 0.9596) from 626.16 to 2088 ha, then decreased and remained around 2000 ha (Figure 19).

’Pinot noir’ surface increased significantly (R^2 = 0.9531) from 694.84 to 2021) (Figure 20). This variety is mainly use for sparkling wines so the increase of the surface of Pinot noir.

’Sauvignon blanc’ increased (R^2 = 0.9264) from 3760.97 to 5632 (Figure 21).

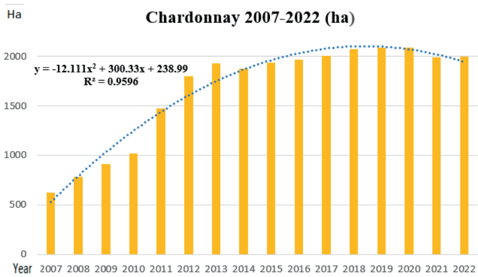

Figure 19. ‘Chardonnay’ surface 2007-2022

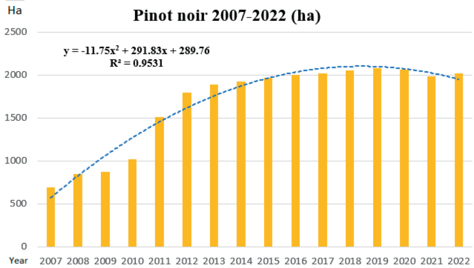

Figure 20. ‘Pinot noir’ surface 2007-2022

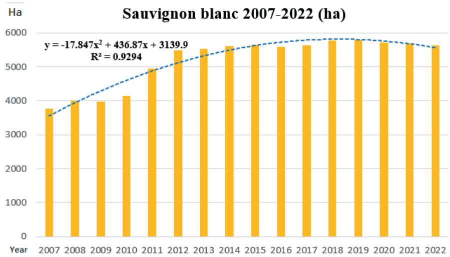

Figure 21. ‘Sauvignon blanc’ surface 2007-2022

’Cabernet Sauvignon’, which increased significantly (R^2 = 0.987) from 3387.16 ha immediately after the start of reconversion to 5452.5 ha, followed by a plateau with almost insignificant variations until the end of the studied period (Figure 22).

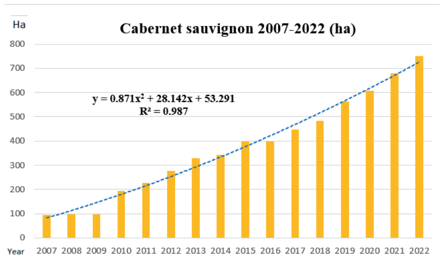

Figure 22. ‘Cabernet Sauvignon’ surface 2007-2022

’Pinot gris’ increased (R^2 = 0.8917) from 1114.72 to 1457 (Figure 23).

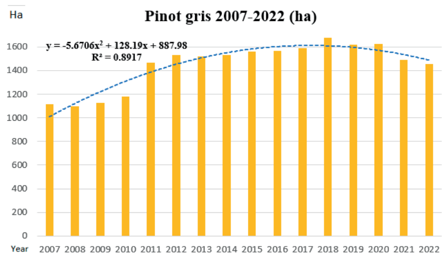

Figure 23. ‘Pinot gris’ surface 2007-2022

’Cadarca’ increased from 51.99 to 98.9 ha, then decreased and remained around 80 ha (Figure 24).

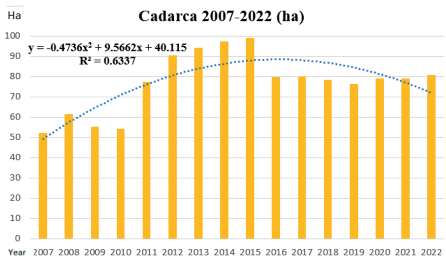

Figure 24. ‘Cadarca’ surface 2007-2022

‘Traminer aromat’ increased from 1.41 to 30.44, with a lot of fluctuations (Figure 25).

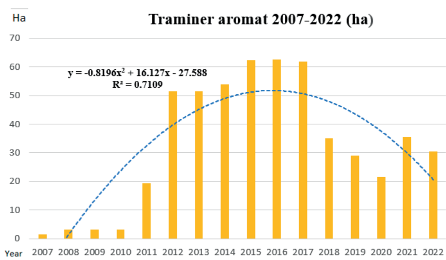

Figure 25. ‘Traminer aromat’ surface 2007-2022

Areas planted to certain wine grape varieties, either international or native, have steadily increased over the 16 years analysed: ‘Busuioaca de Bohotin’ - from 96 to 752.1 ha) (Figure 26), ‘Cabernet Franc’ - from 12.3 to 157.6 ha) (Figure 27), ‘Cramposie selectionata’ - from 0.01 to 20.3 ha, then began a massive and steady growth to 135.4 ha) (Figure 28), ‘Feteasca neagra’ - from 1307.2 to 3205 ha (Figure 29), ‘Negru de Dragasani’ - from 0.33 to 362.19 ha (Figure 30), ‘Riesling de Rhin’ - from 20.61 to 459.98 ha (Figure 31), ‘Syrah’ - from 19.39 to 685 ha (Figure 32), ‘Tamaioasa romaneasca’ from 862.77 to 1757 ha (Figure 33), ‘Traminer roz’ - from 293.55 to 597.1 ha (Figure 34),‘Viognier’ - from 0 to 57.68 ha (Figure 35).

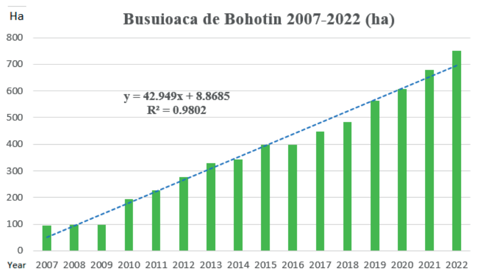

Figure 26. ‘Busuioaca de Bohotin’ surface 2007-2022

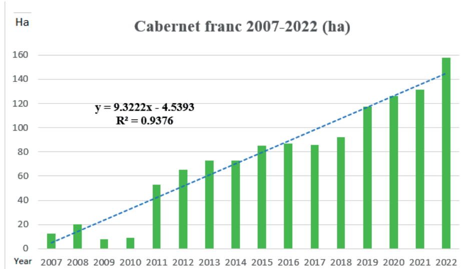

Figure 27. ‘Cabernet franc’ surface 2007-2022

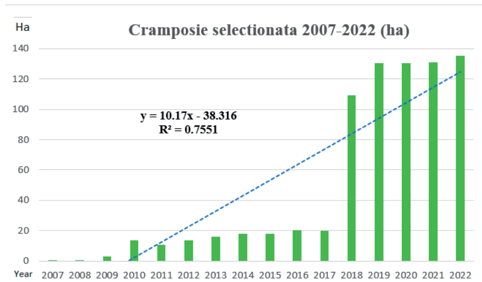

Figure 28. ‘Cramposie selectionata’ surface 2007-2022

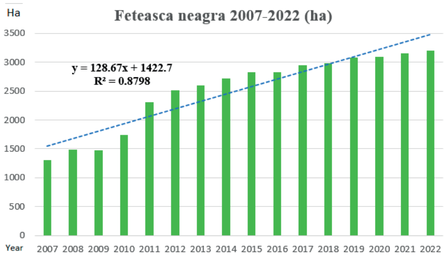

Figure 29. ‘Feteasca neagra’ surface 2007-2022

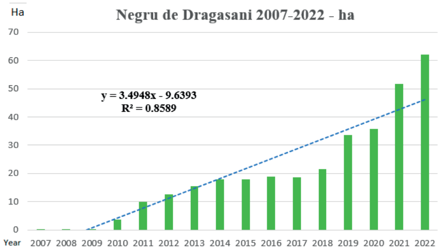

Figure 30. ‘Negru de Dragasani’ surface 2007-2022

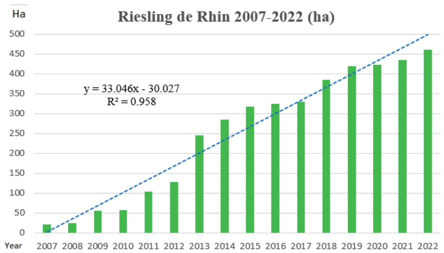

Figure 31. ‘Riesling de Rhin’ surface 2007-2022

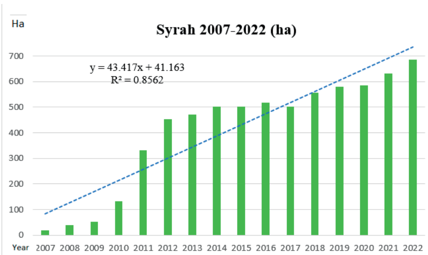

Figure 32. ‘Syrah’ surface 2007-2022

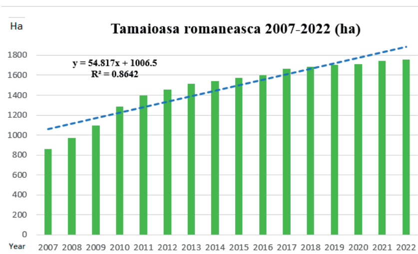

Figure 33. ‘Tamaioasa romaneasca’ surface 2007-2022

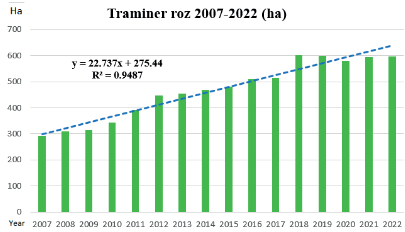

Figure 34. ‘Traminer roz’ surface 2007-2022

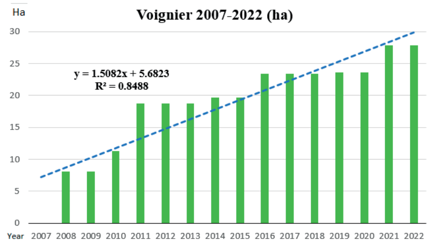

Figure 35. ‘Viognier’ surface 2007-2022

Even though Romania has valuable autochthonous wine varieties, it represent only 20.71% of the total surface cultivated. The Romanian varieties for white wines have a percentage of 17.41%, while the autochthonous varieties for red wines represent only 3.30 % of the total surface cultivated with wine varieties (Cichi et al., 2020).

Some of Romanian varieties such as ‘Mustoasa de Maderat’, ‘Majarca’, ‘Iordana’, ‘Feteasca alba’ and ‘Feteasca regala’ have a descrease during the last 16 years, this state of art being influences by many factors climate change, as shown by the statistically significant increase of temperature, severe heatwaves, drought and aridity and restrictions in agricultural practices in different regions (Bucur & Babeş, 2016; Vlăduț et al., 2023).

The areas cultivated with some varieties (international and indigenous) fluctuated slightly around certain values ’Francusa’ (about 300 ha), ’Furmint’ (about 21 ha), ’Galbena de Odobesti’ and ’Rkatiteli’ (about 400 ha), ’Grasa de Cotnari’ (about 500 ha), ’Neuburger’ (about 70 ha), ’Novac’ (about 73 ha), ’Plavaie’ (about 150 ha), ’Sarba’ (about 270 ha), and ’Zghihara de Husi’ (about 60 ha), during the 16-year period analyzed, while other varieties had only significant increases: ’Feteasca neagra’ (R^2 = 0,8798), ’Negru de Dragasani’ (R^2 = 0,8589), ’Syrah’ (R^2 = 0,8562), and ’Tamaioasa romaneasca’ (R^2 = 0,8642), and

’Cramposie selectionata’ (R^2 = 0,7551) too, stagnated between 2007-2017, followed by a steep rise, so this native wine grape variety is on an uptrend. The relevant increase in the areas planted with Romanian varieties such as ‘Tamaioasa romaneasca’, ‘Busuioaca de Bohotin’, ‘Negru de Dragasani’, ‘Feteasca neagra’ or ‘Cramposie selectionata’, in the period 2007-2022, is mainly due to the marketing strategies of the wineries to exploit and promote local varieties and to bring distinctive products to the international wine market.

In conditions where these native varieties are not sufficiently promoted to be cultivated, the extension of planted areas, ensuring traceability, certification of wines and their participation in international competitions, represent a combination of actions for the preservation of the national genetic heritage of grapevines and the conservation of Romanian resources of high genetic value. ‘Feteasca neagra’, one of the most valuable wine grape varieties in Romania for the production of premium red wines, occupies only 3% of the Romanian wine-growing area and has been steadily increasing over the last 16 years.

CONCLUSIONS

The new investments in Romanian vineyard took into account the necessary increase in wine quality, thus, the new were mostly destined for the PDO wine production. Through the reconversion/ restructuring programs with national and European funds, Romanian vineyards increased their surface by only 4370.9 ha, which means an average of 273.18 ha per year. ‘Negru de Dragasani’, a native Romanian variety for red Premium quality wines had an interesting increase mainly to its high ecological plasticity and marketing strategies of the small wineries, selling specific Romanian wines.

In the current context, there is a real need for a strategic national alliance between government institutions, wine growers and producers, wine market sectors to achieve a better valorisation of areas with a vocation for wine grape production. There is also a need to identify and evaluate Romanian wine grape varieties with high market potential.

ACKNOWLEDGEMENTS

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***HOTĂRÂRE nr. 578 din 9 iulie 2014 privind stabilirea modului de acordare a sprijinului financiar din partea Uniunii Europene pentru producătorii din sectorul vitivinicol în perioada 2014-2018, cu completările ulterioare

***HOTĂRÂRE nr. 868 din 31 octombrie 2018 privind stabilirea modului de acordare a sprijinului financiar din partea Uniunii Europene pentru producătorii din sectorul vitivinicol în perioada 2019-2023, cu completările ulterioare

***ORDIN nr. 211 din 15 martie 2007 pentru aprobarea Normelor de aplicare a programelor de restructurare/ reconversie a plantaţiilor viticole, pentru campaniile 2006-2007 şi 2007-2008, derulate cu sprijin comunitar, cu modificările și completările ulterioare

***ORDIN nr. 247 din 23 aprilie 2008 privind aprobarea Normelor de aplicare a programelor de restructurare/ reconversie a plantaţiilor viticole, derulate cu sprijin comunitar pentru campaniile 2008/2009-2013/2014, cu modificările și completările ulterioare

*** ORDIN nr. 1763 din 10 noiembrie 2014 pentru aprobarea Normelor metodologice privind condiţiile de punere în aplicare a măsurii de restructurare/ reconversie a plantaţiilor viticole, eligibilă pentru finanţare în cadrul Programului Naţional de Sprijin al României în sectorul vitivinicol 2014 – 2018, cu modificările și completările ulterioare

***ORDIN nr. 1508 din 17 decembrie 2018 pentru aprobarea Normelor metodologice privind condiţiile de punere în aplicare a măsurii de restructurare/ reconversie a plantaţiilor viticole, eligibilă pentru finanţare în cadrul Programului naţional de sprijin în sectorul vitivinicol 2019-2023, cu modificările și completările ulterioare

***REGULAMENTUL (CE) NR. 1234/2007 din 22 octombrie 2007 de instituire a unei organizări comune a pieţelor agricole şi privind dispoziţii specifice referitoare la anumite produse agricole ("Regulamentul unic OCP"), cu modificările și completările ulterioare
***REGULAMENTUL (CE) NR. 555/2008 din 27 iunie 2008 de stabilire a normelor de aplicare a Regulamentului (CE) nr. 479/2008 al Consiliului privind organizarea comună a pieţei vitivinicole în ceea ce priveşte programele de sprijin, comerţul cu ţările terţe, potenţialul de producţie și privind controalele în sectorul vitivinicol, cu modificările și completările ulterioare
***REGULAMENT nr. 1150 din 15 aprilie 2016 de stabilire a normelor de aplicare a Regulamentului (UE) nr. 1308/2013 al Parlamentului European şi al Consiliului în ceea ce priveşte programele naţionale de sprijin în sectorul vitivinicol, cu modificările ulterioare
***REGULAMENT nr. 2289 din 21 decembrie 2021 de stabilire a normelor de aplicare a Regulamentului (UE) 2021/2115 al Parlamentului European şi al Consiliului în ceea ce priveşte prezentarea conţinutului planurilor strategice PAC şi sistemul electronic pentru schimbul securizat de informaţii
*** https://www.onvpv.ro/ro/content/legislatie

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ENHANCING OF BIOAVAILABILITY OF VALUABLE COMPOUNDS FROM GRAPE POMACE BY ENZYMATIC TREATMENT

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Abstract

Grape pomace, the most abundant winemaking by-product, represents a possible source of valuable biocompounds that can be valorised in different domains of industry also providing environmental benefits. The investigation performed provides relevant data on the potential of various enzyme mixtures (β-glucanase, hemicellulases, xilanases, pectinases, proteases) to release monosaccharides, amino acids and polyphenols by grape pomace's hydrolysis, thereby increasing its nutritional and functional properties. The reducing sugars, the aminoacids and the polyphenols obtained were determined, and the antioxidant activity was measured before and after the enzymatic treatments of the grape pomace. The researches performed on two varieties of grape pomace indicated that the white variety was noted with a higher quantity of total soluble sugars, while red grape pomace was found to have higher amounts of amino acids, polyphenols and higher antioxidant activity. The enzymatic treatment of grape pomace enhanced the extraction yield by up to 59% for reducing sugars, respectively 55% for polyphenols.

Key words*: antioxidant activity; enzymatic hydrolysis; glucanases; pectinases; polyphenols.*

INTRODUCTION

Grape (*Vitis* spp.), one of the most appreciated fruits, represents a significant crop cultivated all over around the world. Approximately 75% of the entire grape production is utilized for wine-making (Zhu et al., 2015), producing millions of tons of residues, such as grape pomace, which represents 20 to 25% of the total processed grapes. Incineration or storage of grape pomace in landfills results in environmental pollution, and waste management can become a significant problem for wine producers. Efforts are currently being made to valorise grape pomace in different domains of industry as an environmentally sustainable alternative.

Grape pomace mainly consists of grape seeds, skins and stalks. According to Nerantzis and Tataridis (2006) one ton of grape pomace is composed of around 430 kg of grape skins, 250 kg of grape stalks and 230 kg of grape seeds. Seeds are rich in antioxidant compounds, such as phenolic acids, flavonoids and procyanidins. In addition, grape seeds were found to have a high content of oil (between 15 to 18%) which is rich in essential fatty acids, non-digestible carbohydrates, proteins and bioactive compounds such as tocopherols and β-carotene (Fiori et al., 2014). Grape skin and pulp are rich sources of fibers and phenolic compounds depending on the vinification techniques and grape variety considered.

There are numerous studies regarding the use of grape pomace in human nutrition as a source of dietary fiber in bread and biscuits (Mildner-Szkudlarz et al., 2013) or to improve the nutrition profile of frankfurters (Ozvural & Vural, 2011), yoghurt and salad dressing (Tseng & Zhao, 2013). There have also been developed several experimental feeding strategies regarding the use of grape pomace for ruminants (Ianni & Martino, 2020) but also for pigs (Bertol et al., 2017) or poultry (Kumanda et al., 2019).

Beside this, grape pomaces constitute an inexpensive source for the extraction of bioactive compounds that can be used in the pharmaceutical and cosmetic industries (Fontana et al., 2013) due to their potential benefits on health promotion and prevention. However, their efficacy in products may be reduced due to the tight binding to cellulose matrices. Certain mechanisms such as hydrophobic interactions and hydrogen bonds between cell wall polysaccharides and various polyphenols (Lin et al., 2016) were studied in grapes and other plants, therefore the treatment with enzymes (pectinases, cellulases, glucanases) has been performed to release the polyphenols from their matrices in order to enhance their bioavailability and efficacy (Holland et al., 2020).

There are numerous researches investigating the capitalization of grape pomace by extracting bioactive compounds that can be used in the pharmaceutical, cosmetic and food industries.

As enzymes differ in hydrolysis efficacy, the present study was focused on testing some commercial enzymatic complexes based on cellulases, pectinases, glucanases and proteases activity in order to increase the nutritional and functional properties of this by-product through hydrolysis. Enzymatic treatment of white and red grape pomace was performed using commercial enzymatic complexes in different concentrations. The reducing sugar, amino acids and polyphenols obtained were determined, and the antioxidant activity was also measured before and after the enzymatic treatments.

Using grape pomace as valuable source of essential nutrients represents not only a method of valorisation of waste from the wine industry, but also an ecological alternative for their disposal, helping to improve sustainable agricultural practices.

MATERIALS AND METHODS

Substrate

The by-product used in our study was grape pomace from *Vitis vinifera* L., white and red varieties, provided by a Romanian vineyard. To be used in the analysis performed, the grape pomace was ground to increase the accessibility of enzymes to the complex polysaccharides (starch, celluloses, hemicelluloses).

Enzymes hydrolysis

Enzymatic hydrolysis was performed using three commercial enzyme complexes representing combinations of xilanases and β-glucanase (1), pectinases and hemicellulases (2) and proteases (3), that were added to the substrate as a mixture. Concentrations (E.C.) between 0.25% and 1% of the enzyme mixture relative to the reaction medium were tested. The enzymatic treatment was performed for 20 h, at 50°C, pH = 5 to 5.5, on a stirred water bath at a constant stirring rate of 120 rpm. Control samples without enzymes added were considered for all conditions.

Total soluble sugars determination

The determination of total soluble sugars was performed according to the Somogyi-Nelson method described by Iordachescu & Dumitru (1988). First non-reducing sugars were converted into reducing sugars by hydrolysis with hydrochloric acid. The reducing sugars obtained react with alkaline copper tartrate when heated, forming cuprous oxide. This reduces arsenomolybdic acid and forms molybdenum blue. The measurements of absorbance were achieved with a UV/Visible spectrophotometer (ThermoSpectronic Helios) at 510 nm. The results were expressed in g% FW (fresh weight).

Total polyphenols determination

The total polyphenols content was determined according to the modified Folin-Ciocalteu assay (Singleton et al., 1999). The method is based on chemical reduction of Folin-Ciocalteu reagent (a mixture of tungsten and molybdenum oxides) and measuring the intensity of the obtained blue colour at 750 nm. The polyphenols content values were expressed in terms of gallic acid equivalent, which is a common reference compound (mg GAE/100 g FW).

Free amino acids determination

The determination of the amino acids content was performed using the ninhydrin assay (Suna et al., 2006), a spectrophotometric method based on the reaction between amino acids and ninhydrin, resulting in the development of a purple-coloured compound known as Ruhemann's purple. The colour intensity of the formed complex is proportional to the concentration of amino acids in the solution, so absorbance was measured at 570 nm. The results were expressed in g% FW.

DPPH scavenging assay

Total antioxidant capacity (radical scavenging activity) was determined using the stable free radical DPPH (diphenylpicryl-hydrazyl) method according to the procedure adapted by Brand-Williams et al. (1995) for complex matrices. 2 ml of 100 μM solution of DPPH in methanol were mixed with 1 ml of different concentrations of grape pomace extract in 80% aqueous methanol. The absorbance (A) of the reaction mixture was measured at 515 nm after 30 min incubation in dark at room temperature. The percentage of the radical scavenging activity (RSA) was calculated according to the formula:

$$\% \text{ RSA} = (1-[A_{sample}/A_{control\ t=0}])100^{-1}$$

DPPH solution in 80% methanol was used as a control. The IC_{50} parameter (mg/ml) for each sample, defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals, was calculated from the linear regression curve of the sample extracts against the percentage of the radical scavenging activity.

Statistical analyses

All measurements were carried out in triplicate, and the results are presented as means ± standard deviation (SD). The significance of the influence of the enzymatic treatment on the measured parameters was assessed by one-way ANOVA test for means discrimination at a confidence level of 95% ($P < 0.05$) using Microsoft Excel Office 2019 for Windows.

RESULTS AND DISCUSSIONS

The chemical composition of grape pomace can vary depending on factors such as grape variety, obtaining method, climate or location. High variation in grape pomace composition was found considering that fermentation of the whole grape mass is required for making red wine, while the rose and white wines are made by juice fermentation, therefore multiple ways of grape pomace capitalization are possible (Antonic et al., 2020).

Three commercial enzymatic complexes containing carbohydrases and proteases were tested in this study with the aim of enhancing the yield of chemical compounds extraction from grape pomace. The hydrolysis degree of complex sugars, the release of amino-acids and polyphenols and the antioxidant capacity of grape by-products after enzymatic reaction with carbohydrases and proteases for 20 h were evaluated.

Influence of hydrolysis treatment on the total soluble sugar content

An enzymatic mixture containing a combination of carbohydrases (complex 1, containing xilanases and β-glucanase; complex 2, containing pectinases and hemicellulases) in a 1:1 ratio was tested on white and red grape pomaces. The total sugars content was determined in grape pomaces samples after treatment with concentration varying between 0.25% and 1% of the enzymatic combination compared to control samples (with no enzyme added).

The analysis performed on the control samples indicated a lower reducing sugars content in red grape pomace (0.20 ± 0.01 g%) compare to white grape pomace (2.82 ± 0.06 g%) (Figure 1). Recent studies have found that the content of the simple sugars in red grape pomaces is usually low due to the difference in red and white wine production technology. Jin et al., in the year 2019, found that the total sugar content was in the range of 7.79 to 14.3 g/kg DW for red grape pomace, while higher amounts of soluble sugars were present in white grape pomace (69.8 g/kg, 75.2 g/kg, and 107.6 g/kg DW for Petit Manseng, Viognier, and Vidal Blanc pomace, respectively).

White grape pomace is obtained by pressing grapes for juice, while red grape pomace is collected after the fermentation of grape pulps for several days. During this process, most sugars are consumed by yeast cells (Jin et al., 2019; Antonic et al., 2020).

The high amounts of sugars remaining in white grape pomace can be recovered and used in various fields, such as for the production of aldonic acids, used in the cosmetics and plastic industry (Tomaszewska et al., 2018) or for fermentation to produce biochemicals or biofuels (Corbin et al., 2015).

Adding a combination of carbohydrases to grape pomace, degrading the cell wall polysaccharides, increased the content of reducing sugars.

The results obtained in this study revealed that the enzymatic treatment was more effective on the red grape pomace, resulting in about 59% increase of the amount of reducing sugars content compared to the control samples (with no enzymatic treatment) (Figure 1). In the case of white grape pomace, the 0.5% concentration of the enzyme complex led to obtaining the highest amount of released sugars, but the increase in reducing sugar content after enzymatic treatment was lower (26%) compared to the red pomace.

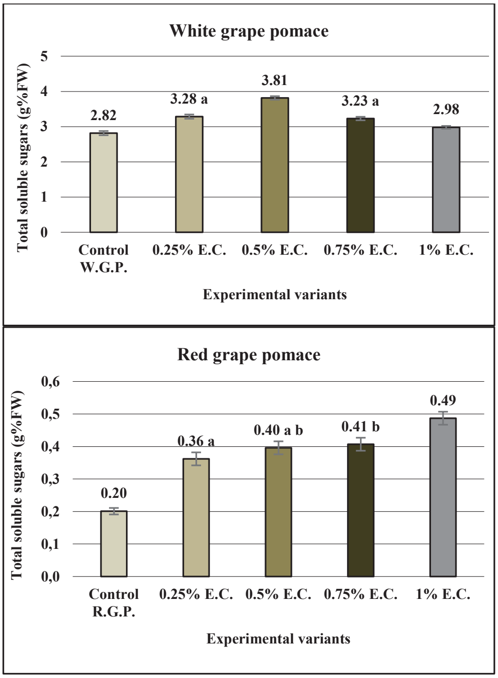


Figure 1. Total soluble sugars content in the grape pomace samples
Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

These results provide data about the potential of some commercial enzymatic complexes on the release of reducing sugars from grape pomace, improving their nutritional value, in accordance with the results found in other studies (Chamorro et al., 2012; Alberici et al., 2020). For example, Chamorro et al. (2012) found that the addition of carbohydrases to grape pomace, either alone or in combination, degraded the cell wall polysaccharides, increasing the content of monosaccharides.

Influence of hydrolysis treatment on the amino acids content

Enzymatic complexes containing proteases were tested on white and red grape pomace. The amino acids content was determined in grape pomace samples after enzymatic treatment with concentration varying between 0.25% and 1% of enzymatic complex compared with control samples (with no enzyme added).

In general, there is more information available about the protein content of grape pomace than about the amino acid content in this by-product. Thus, the crude protein content reported ranged from 6.3 g% to 10.6 g% in white grape pomace, while the same parameter ranged from 11.5 g% to 13.0 g% in red grape pomace (Jin et al., 2019; Marin et al., 2020). However, some authors found that in dry grape marc with seeds or without seeds have similar amounts of essential amino acids as those found in cereals: for instance, lysine ranges 0.544 g% in the seed-less grape marc, higher than the values reported for corn (0.261 to 0.399 g%) and wheat (0.352 to 0.395 g%) (Olteanu et al., 2014).

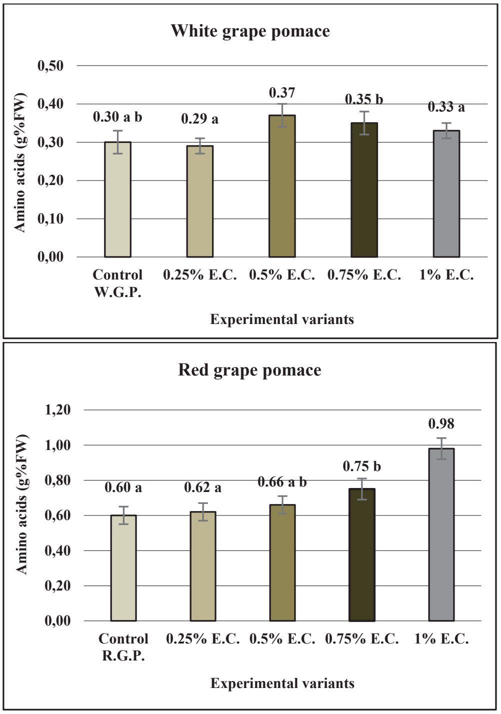


Figure 2. Amino acids content in the grape pomace samples
Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

The research performed in this study (Figure 2) found a higher content in free amino acids in red grape pomace (0.6 ± 0.05 g%) compare to that from white pomace (0.3 ± 0.03 g%) in the control samples. The enzymatic treatments resulted in a low increase of the amino acids content in the white pomace, with around 19% more amino acids determined in the sample with the highest enzymes concentration added compare to the control. In contrast, the red pomace samples had 39% more amino acids after enzymatic treatment compared to the control.

Influence of hydrolysis treatment on the total polyphenols content

In recent years grape pomace is frequently used as a source of polyphenols. Because of an incomplete extraction during the winemaking process, grape pomace contains high amounts of phenolic compounds, which are known as secondary plant metabolites with potential beneficial effects on human health due to their antioxidant activity.

In this study, the same enzymatic mixture containing a combination of carbohydrases as described for the sugar determination was tested on white and red grape pomaces regarding polyphenols content. The total polyphenols content was determined in grape pomace samples after treatment with concentration varying between 0.25% and 1% of enzymatic mixture compared with control samples (with no enzyme added).

The grape pomace varieties tested showed high but diverse contents of polyphenols. Red pomace had 1.8 times more polyphenols (365.21 ± 3.40 mg GAE/100 g FW) than white pomace (203.42 ± 8.53 mg GAE/100 g FW) before the enzymatic treatment (Figure 3). Also, Jin et al. (2019) found differences between white and red grape pomace after a comparative evaluation of polyphenols content, which ranged from 11.8 to 32.1 g/kg DW gallic acid equivalents in white grape pomace and from 10.4 to 64.8 g/kg DW gallic acid equivalents in red grape pomace.

The amounts of polyphenols in the extracts after enzymatic treatment showed an important increase of 55% in the case of white pomace and less significant in the case of red pomace (increase of 13%). The effect of enzymatic concentrations used in the experiment was found to be dependent on the grape variety. White grape pomace was found to be more sensitive to enzymatic action with the highest polyphenols yield (446.65 ± 17.13 mg GAE/100 g FW) obtained when white pomace was treated with the maximum concentration (1%) of enzymatic mixture.

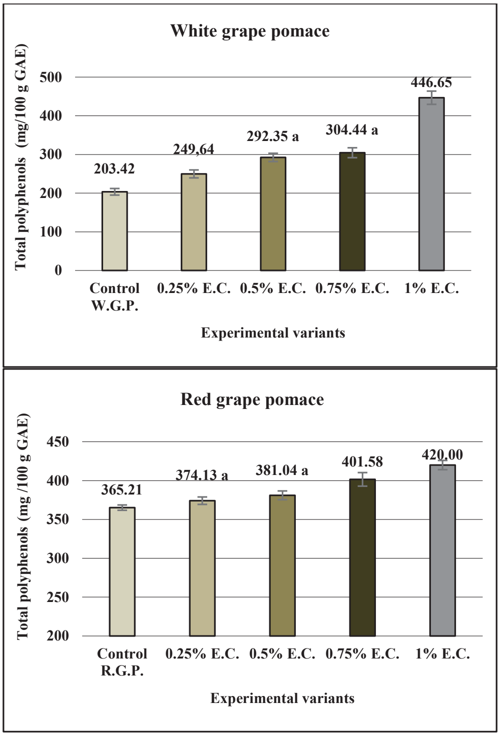


Figure 3. Total polyphenols content in the grape pomace samples

Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

Starting from the hypothesis that enzyme degradation of plant cell wall polysaccharides can potentially enhance the release of bioactive phenolics, also Chamorro et al., in the year 2012, and Meini et al., in the year 2019, found that pectinases and cellulases were effective in releasing polyphenolics bound to the cellulose matrices of grape skins and seeds. According to Meini et al., the enzymatic treatment enhanced the extraction yield of phenolics by up to 66% and its antioxidant capacity by up to 80%. Thus, they found that tannase increases the antioxidant capacity of the extract by releasing of gallic acid, while cellulase favours the releasing of malvidin-3-O-glucoside and p-coumaric acid.

Influence of hydrolysis treatment on the antioxidant capacity

Antioxidant capacity (radical scavenging activity) of grape pomace varieties was evaluated in the same samples used for polyphenols determination and the IC_{50} values (the concentration of sample that is required to scavenge 50% of DPPH free radicals) were calculated for further comparison (Table 1).

The measurements performed before the enzymatic treatment indicated that the highest antioxidant activity was found in red pomace (4.65 mg/ml expressed as IC_{50} value). The lowest scavenging capacity was recorded in the white pomace, which required therefore a higher concentration (11.43 mg/ml) to scavenge 50% of DPPH free radicals compared to red pomace.

After enzymatic treatment, the DPPH radical scavenging activity was found to be higher in the grape pomace samples compared to untreated grape pomace. The increase in antioxidant activity was more pronounced in the white grape variety (2.21 times higher) than in the red variety, where enzymatic treatment determined an increase of only 1.35 times of the antioxidant activity in relation to the control sample.

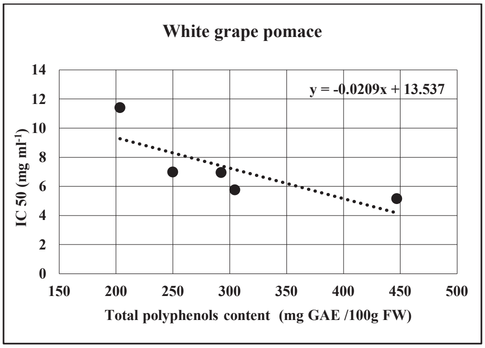
Table 1. IC_{50} values of DPPH scavenging activities in the grape pomace samples

Grape pomace variety	Experimental variants	IC_{50} (mg/ml)
White	Control (0% E.C.*)	11.43
	0.25% E.C.	7.00
	0.5% E.C.	5.77
	0.75% E.C.	5.17
	1% E.C.	6.97
Red	Control (0% E.C.)	4.65
	0.25% E.C.	4.15
	0.5% E.C.	3.44
	0.75% E.C.	3.76
	1% E.C.	4.05

*E.C. = enzymatic concentration in the reaction mixture

Antioxidant activity shows the same tendency as the phenols content, most likely due to the fact that increasing amounts of phenols results in a higher free radical scavenging. The relationship between the IC_{50} values and total polyphenols contents was performed via Pearson's correlation coefficient (R). The variables polyphenols content and IC_{50} measured at the tested enzymatic concentration showed a good correlation for red grape pomace (R = 0.9580) (Figure 4), but a weaker correlation was calculated for the white grape pomace (R = 0.7798).

Also, Chamorro et al., in the year 2012, found that enzymatic treatment with pectinase facilitated the release of gallic acid from grape pomace increasing the antioxidant activity.



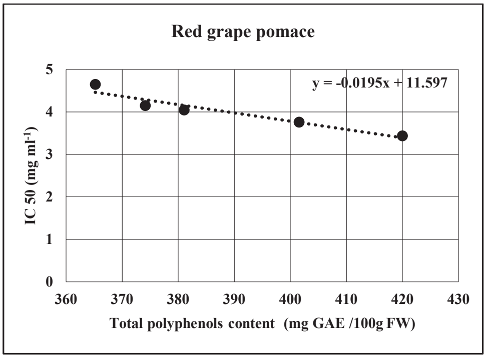


Figure 4. Correlation between total polyphenols content and antioxidant activity

The valuable content in bioactive compounds of grape pomace can have multiple applications. Further research indicated that due to its high antioxidant activity, grape pomace polyphenolic extract can be used as a replacement of synthetic antioxidants in food products in order to obtain the extension of its shelf-life (Dodan et al., 2021).

CONCLUSIONS

Grape pomaces, the most abundant winemaking by-product, represent a potential source of valuable biocompounds which can be recovered and used in various fields. The comparative study of the tested grape pomace varieties indicated that the white variety was noted with a higher content of total soluble sugars, while red grape pomace was found to have a higher content of amino acids, polyphenols and higher antioxidant activity.

The research performed also provided relevant data about the potential of different enzymes combination (xilanases, β-glucanase, pectinases, hemicellulases, proteases) to release monosaccharides, amino acids and polyphenols from grape pomace, which could improve the nutritional value and the antioxidant capacity of the by-product.

Reusing grape pomace as a nutritional supplement both for human and animals or as functional ingredients in the pharmaceutical and cosmetic industries would provide a sustainable alternative for recovering and valorizing the wine industry by-product, while also promoting environmental advantages.

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BIOLOGICAL ASPECTS AND MANAGEMENT APPROACHES OF THE GRAPEVINE PEST, *PULVINARIA VITIS*, IN THE CLIMATE CHANGE CONTEXT

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Abstract

The aim of this study arises from the emergence of new outbreaks producing damage in vineyards from Transylvania and also from the limited amount of updated literature on Pulvinaria vitis, in Romania. The present paper provides an overview of the biology and control of Pulvinaria vitis in the climate change context, because further research is needed, especially on specific regions where it was identified, in order to understand the full range of impacts and to elaborate effective management strategies. In the last few years, wine growers reported the presence of a sticky black residue on the grapevines and a cottony grape scale, which seems to produce economic losses. Pulvinaria vitis has the ability to cause damage by feeding on phloem sap and excreting honeydew on which sooty mold develops, reducing the plant's capability of respiration, transpiration, and photosynthesis. Additionally, it has been identified as a vector for several grapevine-infecting viruses. Recent climate change appears to be influencing the distribution and life cycle of Pulvinaria vitis and also making the plants more susceptible to pest damage.

***Key words**: climate change, vineyard pest, management strategies, Pulvinaria vitis*

INTRODUCTION

At the global level, the last two years were characterized by high rates of inflation, disruptions in supply chains and an energy crisis induced by the Ukraine conflict. In this context, many markets experienced substantial increases in wine prices which lead to a slight decline in global consumption volumes. The current value of international wine exports, though, is at an all-time high (State of the world vine and wine sector in 2022. April 2023. OIV. Retrieved from https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf), therefore the importance of viticulture is increasing.

FAO and OIV latest statistics regarding the current status of grapevine cultivated areas and grapes production in Romania are presented in Figure 1. According to the FAO's statistics, in 2022, Romania harvested an area of 159.740 ha (representing 2.6% of the global harvested area) and produced 804.800 tons of grapes (Food and Agriculture Organization of the United Nations. Crops and livestock products. Data retrieved from www.fao.org/faostat/en/#data/QCL). The EU holds 46.20% of the total grape harvested area in the world, and Romania ranks 5th out of the 25 EU member states. OIV supports the FAO`s statistics and ranks Romania on the 10th place out of the 98 states of the world (Country Statistics. International Organisation of Vine and Wine. Romania, 2022 data. Retrieved from https://www.oiv.int/what-we-do/country-report?oiv). Also, OIV statistics highlight a 0.6% decrease of the harvested area in Romania in 2022 compared to 2021. In fact, significant decreases in the harvested areas are noted for the last 2 decades all over the world. Even so, the production of grapes has the tendency to remain at the same levels (World Statistics. International Organisation of Vine and Wine. World, 2022 data. Retrieved from https://www.oiv.int/what-we-do/country-report?oiv). This implies that along time, better vineyard management strategies were used and this domain is always under ongoing adjustments and improvements.

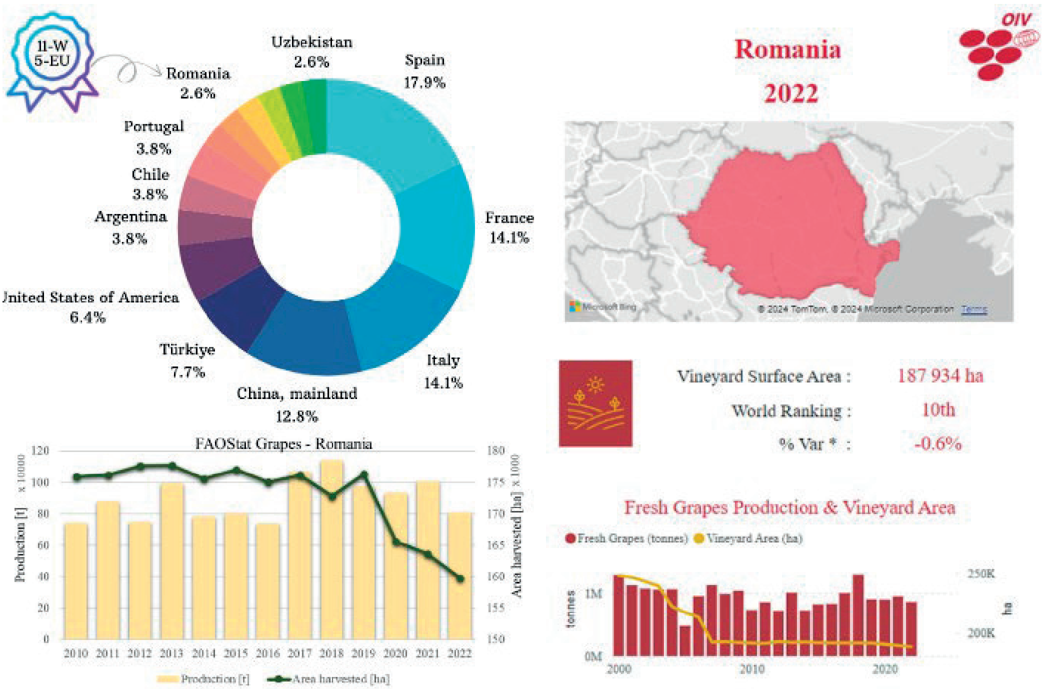

Figure 1. Statistics regarding the harvested areas and grape productions in Romania

Due to its fertile soils and advantageous climatic conditions, viticulture has thrived in Romania since ancient times (Chedea et al., 2021; Chiurciu et al., 2020; Muntean et al., 2022). Positioned in Eastern Europe, Romania spans from 43°37′ to 48°15′ N latitude and 20°15′ to 29°44′ E longitude. The country's climate is classified as temperate continental according to the updated Koppen-Geiger climate classification, supporting eight prominent viticultural regions (Irimia et al., 2018; Chedea et al., 2021; Muntean et al., 2022). In the last decade the cultivation of grapevines in Romania, deeply rooted in history and tradition, is transforming due to climate change. Even if this phenomenon is reshaping the viticultural landscape, Romanian viticulture has shown adaptability and resilience to changes in climate over time (Brașoveanu et al., 2020). Statista Research Department reports that approximately 1.2 thousand hectares of Romania's vineyards were under reconstruction and conversion in 2023 (Statista, 2023).

In a report from 2022, The Intergovernmental Panel on Climate Change states that the occurrence of hazards is on the rise due to climate change, which increases susceptibility and exposure and reduces the capacity of systems and individuals to cope. In addition to crop and agricultural production losses, the repercussions include the destruction of agricultural biodiversity balance and negative chain reactions that have far-reaching ramifications on a local, national, regional, and even global scale (Pörtner et al., 2022).

A significant aspect of the environmental impact on viticulture is the management of diseases and pests, which may cause pollution (Komárek et al., 2010) and adverse effects on human health (Ntzani et al., 2017). The biology and populations of pests are impacted by climate change; therefore, plant protection measures in vineyards may undergo modifications in response to the escalation of pest or diseases in the vineyards (Caffarra et al., 2012; Salinari et al., 2006). Also, the phenology of the vine, which is significantly impacted by global warming, increases plant vulnerability to pathogens, and vectors behaviour must all be considered in disease and pest management (Reineke & Thiéry, 2016). Adding to all of this the fact that the European Green Deal Agenda includes the reduction of insecticide use as a critical component for its ambitious goal of

making the European Union climate neutral by 2050 (Tataridas et al., 2022), finding the appropriate management strategy could be quite a challenge in the next years.
Observations over the past thirty years regarding pests affecting grapevines have revealed an expansion in the habitat of grapevine mealybugs (Santos et al., 2020), which are part of superfamily Coccoidea, the same as *Pulvinaria vitis*. The Coccoidea forms a significant part of the vine insect complex. In 1997, Pellizzari reports that the soft scales that are identfied as vine pests are *Parthelonecanium persicae* (Fabricus)*, Neopulvinaria innumerabilis* (Rathvon)*, P. corni* (Bouchr)*, and Pulvinaria vitis* (Linnaeus) (Pellizzari G, 1997).
Pulvinaria vitis (Coccoidea superfamily, Coccidae family, *Pulvinaria* genus) also named the cottony grape scale is a scale insect, part of Coccidae family, which are sap-feeding insects. They target the fruit, roots, stems, leaves and buds of the plant. Usually abundant in warmer areas, many species represent important economic pests. The Coccoidea or scale-insects, including *Pulvinaria vitis* have been understudied in Romania and is quite likely that the economic importance of this group is underestimated (Fetykó et al., 2010). It has been estimated that scale insect-related agricultural losses and additional production expenses surpassed, at a global level, 1 billion $ annually (Kosztarab, 1977).
This review aims to explore the biological aspects and management approaches of the grapevine pest, *Pulvinaria vitis,* within the context of climate change, by synthesizing current knowledge and identifying future research needs.

BIOLOGY AND ECOLOGY

Scale insects are often small and easily overlooked therefore, they present considerable challenges regarding the taxonomy, especially due to their complex life histories and morphological variability. Sanders (1909) was among the first to point out the uncertainties and errors in identifying soft scales, by pointing out the influence of the host plant on the development of the pest. Phillips (1962) indicates a complex interaction between genetic factors and environmental conditions, especially the host plant, in determining the morphology of *Pulvinaria* species.
Recent taxonomic clarifications provided by (Tanaka & Kamitani, 2020), offer essential foundations for accurate pest identification and management, by enhancing taxonomic resolutions through the integration of molecular tools with traditional morphological approaches, facilitating precise monitoring and management of *Pulvinaria vitis*. Yet, they also underscore the necessity for continuous refinement of taxonomic frameworks and methodologies.

Taxonomy and description

First described by Linnaeus in 1758, *Pulvinaria vitis* belongs to the phylum Arthropoda, which includes invertebrates with an exoskeleton, segmented body, and jointed appendages. In this phylum, *Pulvinaria vitis* is classified under the class Insecta. The order Hemiptera, to which it belongs, includes insects commonly known as true bugs, most of them being characterized by their piercing-sucking mouthparts. Just like aphids, whiteflies and jumping plant lice, they are members of the Sternorrhycha suborder (Gullan & Cook, 2007). Within this suborder, *Pulvinaria vitis* is further classified into the superfamily Coccoidea, family Coccidae, which includes more than 1200 known species. The Coccidae family is the third biggest in the Coccoidea superfamily (Ben-Dov, 1993) and the *Pulvinaria* genus, within this family, encompasses a group of scale insects, with *Pulvinaria vitis* being a specific species within this genus.
Scale insects are sap-sucking parasites, with different hosts plants almost all over the world. Scales are significant agricultural pests and can be distinguished from other insects by their protective waxy exudate, representing their characteristic appearance (Kozár et al., 2013). In the field of entomological taxonomy, the clarification of synonymies among species names is essential for the accurate identification and study of pests, regarding the case of *Pulvinaria vitis*, Newstead (1903) was the first to publish a synonymy consolidating *Pulvinaria betulae*, *Pulvinaria carpini*, and *Pulvinaria oxycanthae* under the name *Pulvinaria vitis*, thereby establishing priority for this nomenclature over others published simultaneously (Malumphy, 1991). This

taxonomic decision has since directed the nomenclature usage across various regions, with *Pulvinaria vitis* being the preferred term in Western Europe, North America, North Africa, and New Zealand, while *Pulvinaria betulae* was predominantly used in Eastern Europe and Japan (Malumphy, 1991).

In 1909, J.G. Sanders highlighted widespread uncertainty and errors in the classification and identification of soft scales, attributing this confusion primarily to the fact that early entomologists underestimated the fact that the host plant can affect development, size, color and markings of mature females and also to the fact that it was believed that Coccidae insects are sedentary and could not live on different genera of host plants (Sanders, 1909). He disputed the distinct classification of *Pulvinaria innumerabilis* in the U.S., arguing no significant difference from *Pulvinaria vitis* based on a study that showed host plants affect development, size, coloration, and markings of mature females (Sanders, 1909). The publication of Steinweden from 1929, also provides a comprehensive analysis of various Coccidae family genera, including *Pulvinaria vitis*. He describes the morphological characteristics of adult females that differentiate them from other species (Steinweden, 1929).

J. H. H. Philips reported in 1962 four species of scale insects belonging to the genus *Pulvinaria*, identified in a study conducted in peach growing regions of southern Ontario: *Pulvinaria vitis* (Linnaeus)*, Pulvinaria innumerabilis* (Rathov)*, Pulvinaria acericola* (Walsh and Riky) and *Pulvinaria floccifera* (Westwood). Of these only *Pulvinaria vitis* and *Pulvinaria innumerabilis* were present in a number of economic importance. Also, he highlights the fact that the similarity of these two species, particularly in the presence of the ovisac, makes them prone to confusion (Phillips, 1962).

While the provided classification is accurate and valuable, there was still a need for future studies that should aim to incorporate genetic insights to further elucidate the taxonomy and evolutionary biology of *Pulvinaria vitis*. With the scales being small insects, frequently overlooked it is necessary to integrate the taxonomic information with current genetic and molecular studies to refine the understanding of *Pulvinaria vitis*'s position within the Coccidae family. Valuable works, such as those of Hodgson (1994) and Gullan and Martin (2003), highlighted the importance of integrating morphological taxonomy with molecular data to address the complexities of scale insect classification and phylogeny. This approach not only aids in clarifying taxonomic ambiguities but also enhances the comprehension of evolutionary relationships and adaptation mechanisms among scale insects (Gullan & Martin, 2009; Hodgson & Peronti, 2012).

The recent studies of Tanaka and Kamitani (2020) have shed some light on the detailed morphology of *Pulvinaria vitis*, by providing an extensive review of the morphological characteristics of four species of the *Pulvinaria* genus from the Ryukyu Islands, Japan (Tanaka & Kamitani, 2020).

Regarding the scale insect fauna of Romania, Fetyko et al. (2010) stated that it is still poorly known, the first Romanian checklist of scale insects being compiled by Savescu in 1985 (Fetykó et al., 2010). The synthesis of Savescu (1983) validated the presence of *Pulvinaria vitis* in Romania among other two *Pulvinaria* species: *Pulvinaria floccifera* and *Pulvinaria regalis* (Paraschiv, 2023). Teodorescu I. (2018) reported that *Pulvinaria vitis* was also reported in Romania under the synonim *Pulvinaria betulae* (Munteanu, 2018).

Morphology and life cycle

There is a complex interaction between genetic factors and environmental conditions in shaping the morphology of *Pulvinaria* species (Phillips, 1962). The studies and observations made by Sanders (1909), Phillips (1962), and Steinweden (1929), collectively contribute to the understanding of host specificity, and morphological variations within the genus *Pulvinaria* and the broader family Coccidae. Steinweden (1929) experiment, which involved transplanting juvenile larvae of various *Pulvinaria* species between different host plants, revealed significant host-dependent variations in the development of these pest insects. The findings indicated that the host plant plays a crucial role in determining the size, pigmentation, and markings of mature females, suggesting a high degree of phenotypic plasticity within these species (Sanders, 1909). Phillips (1962) work further supports this by

highlighting the minimal variation in specific body elements despite significant differences in overall body size among individuals from different hosts. They all highlight the importance of detailed morphological and ecological studies in understanding the taxonomy, evolution, and host specificity of scale insects (Sanders, 1909; Steinweden, 1929; Phillips, 1962).
J. P. Malumphy (1991) thesis is a valuable investigation work about *Pulvinaria vitis* complex in Europe. A more recent thorough analysis of *Pulvinaria* species from Japan, including *Pulvinaria vitis*, as a type species of the genus, was carried out by Tanaka (2020) and Tanaka & Kamitani (2020). In their study, they provide a detailed morphological description of the adult female of *Pulvinaria vitis*, comparing it with previous descriptions and other species within the genus. Their research provides important new insights into the morphological properties that differentiate *Pulvinaria vitis*, including body size, shape, dorsal and ventral surface structure. In order to distinguish *Pulvinaria vitis* from other species in the genus, a table of diagnostic morphological character sets and a key to the species of *Pulvinaria* are also included. They concluded that morphological descriptions of regional populations are important in order to understand the range of morphological variation in widespread species (Tanaka & Kamitani, 2020).
The life cycle and biological characteristics of soft scales, in general, is challenging due to the presence of great variations among species within the same genus (Kosztarab, 1996). The majority of the species under this group display cryptic characteristics and invasive behaviour (Malumphy, 1991; Paraschiv, 2023). In biparental species, males have a derived form of incomplete metamorphosis, which consists of two feeding nymphal instars followed by the nonfeeding prepupal (third-instar), pupal (fourth-instar), and adult (Camacho & Chong, 2015).
The soft scale insects are known for their distinct morphological features. Typically, the length of the scales does not surpass 5 mm and their sexual dimorphism is evident. The females are usually wingless and their reproduction is frequently parthenogenetic (Malumphy, 1991; Paraschiv, 2023).

According to (Jansen, 2000), the soft scale species of the genus *Pulvinaria* can be distinguished from other genera of the family Coccidae by the combination of specific characters. Immature females are almost twice as long than wide, the tibia and tarsus usually are freely articulating, also they produce a typical white cottony ovisac under the body (Jansen, 2000). The significant microscopic characters are represented by the three spiracular setae of which the median one is two or three times longer than the lateral two and different in shape (Jansen, 2000). Females of most *Pulvinaria* species are oval shaped, flat, wrinkly and the colour is from chestnut to greyish brown (Jansen, 2000).

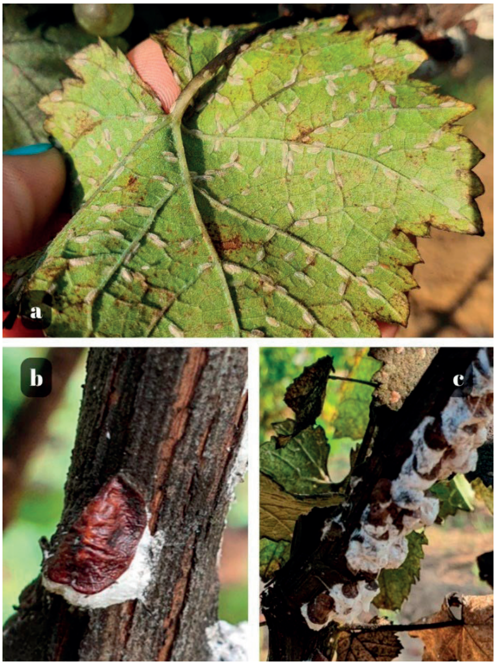

Figure 2. *Pulvinaria vitis* (L2) nymphs on grapevine leaf (a); *Pulvinaria vitis* adult female specimen on grapevine (b); *Pulvinaria vitis* infestation on grapevine (c)

The adult females of *Pulvinaria vitis* are 3-8 mm long, ovoid to circular, dark brown and flat, presenting 3-5 transversal striae. To the post reproductive female, the dorsum gets wrinkled and strongly sclerotized. The females of *Pulvinaria vitis* are known for their ability to produce a cottony ovisac that protects their eggs, a characteristic that is distinctive to the genus and critical for the protection of the next generation (Phillips, 1962).The ovisac is wide convex, cottony, white, and longer than the body of adult female (Kosztarab & Kozár, 1988; Masten Milek et al., 2009). Figure 2 presents morphological aspects of different life stages of *Pulvinaria vitis* on grapevine.

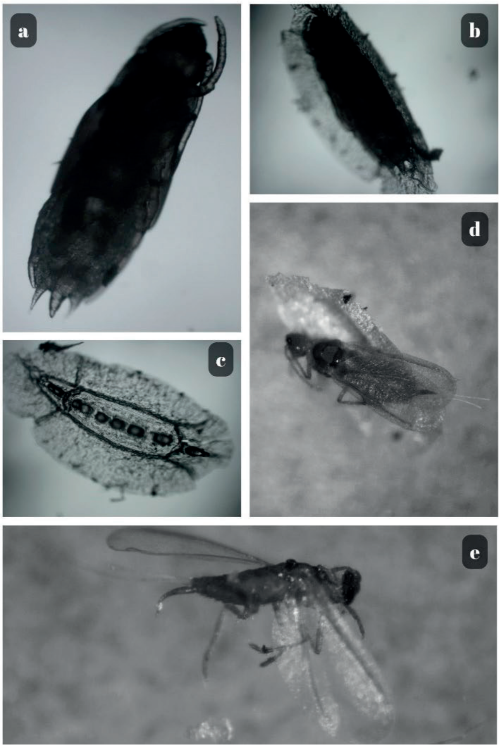

Figure 3. *Pulvinaria vitis* male specimen on different development stages (a, b, d, e); empty male test (c)

The adult males are pinkish and winged (Figure 3), smaller than the females, approximately 1.5 mm long (Gill, 1988; Hommay et al., 2021; Phillips, 1962; Tomoiagă Liliana, 2006). They do not feed as adults and they develop from unfertilized eggs, while females develop from fertilised eggs (Malumphy, 1991). They die after mating in the fall, while females overwinter and then produce white waxy egg masses from the end of April to early June. One adult female produces between 1000 and 5000 eggs, embedded within a cotton-like structure, called the ovisac, which grows beyond the shield and lifts it progressively (Phillips, 1962, 1963; Malumphy, 1991; Giuseppina Pellizzari, 1997; Hommay et al., 2021).

Pulvinaria vitis exhibits an univoltine life cycle, with one generation per year. The reproduction is both sexually and parthenogenetically and the mechanism that regulates the mode of reproduction is not completely understood (Phillips, 1963; M. Kosztarab & F. Kozár, 1988; Pellizzari, 1997; Camacho & Chong, 2015). Adults mate from September to October, then the insect overwinters as young females, protected under the bark of the shoots and completes its development in the following year (Phillips, 1963; Pellizzari, 1997; Camacho, 2015; Jansen, 2000; Hommay et al., 2021).
The eggs are orange to wine-red, 0.20-0.30 mm long, and hatch from late May to June (Tomoiagă, 2006; Hommay et al., 2021) when the temperature reached 14°C. An increase in temperature was found to be associated with an increase in the hatching rate. After the nymphs hatch, they disperse onto the leaves, petioles, or shoots (Phillips, 1963; Pellizzari G, 1997; Hommay et al., 2019). The nymphs, referred to as crawlers, represent the primary dispersal phase, facilitating the colonization of new host plants (Phillips, 1962; Malumphy, 1991). After emerging, the nymphs have phototactic and photokinetic attributes and their establishment to the host plant is influenced by several environmental factors like light intensity, humidity, and airflow (Phillips, 1963).
Pulvinaria vitis presents three nymphal stages with the first moult in July and the second in August (Pellizzari, 1997; Hommay et al., 2021). The first instar is initially just a bit bigger than the egg, around 0.5 mm, but grows rapidly to a length of maximum 1 mm. Initially having a deep orange hue, it transforms into a transparent orange-yellow as soon as it settles on the plant. The first moult takes place 12 to 18 days after hatching, the second after 28 to 36 days, and the third 56 to 93 days after hatching (Philips, 1963). The third instar is described to have maximum 3 mm in length and a yellow to greyish-brown color with reticulate dark brown patterns (Phillips, 1962; Steinweden, 1929; Pellizzari, 1997; Tomoiagă, 2006; Hommay et al., 2021).
A correlation between the life cycle of *Pulvinaria vitis* and the annual life cycle of grapevine is essential in the process of establishing and implementing Integrated Pest Management strategies. In this sense, Figure 4

presents the life cycle of *Pulvinaria vitis* - adapted after Jansen (2000) in correlation with the annual life cycle of grapevine, as described by Venios et al. (2020) Both studies are from the Northern emisphere (from Europe).

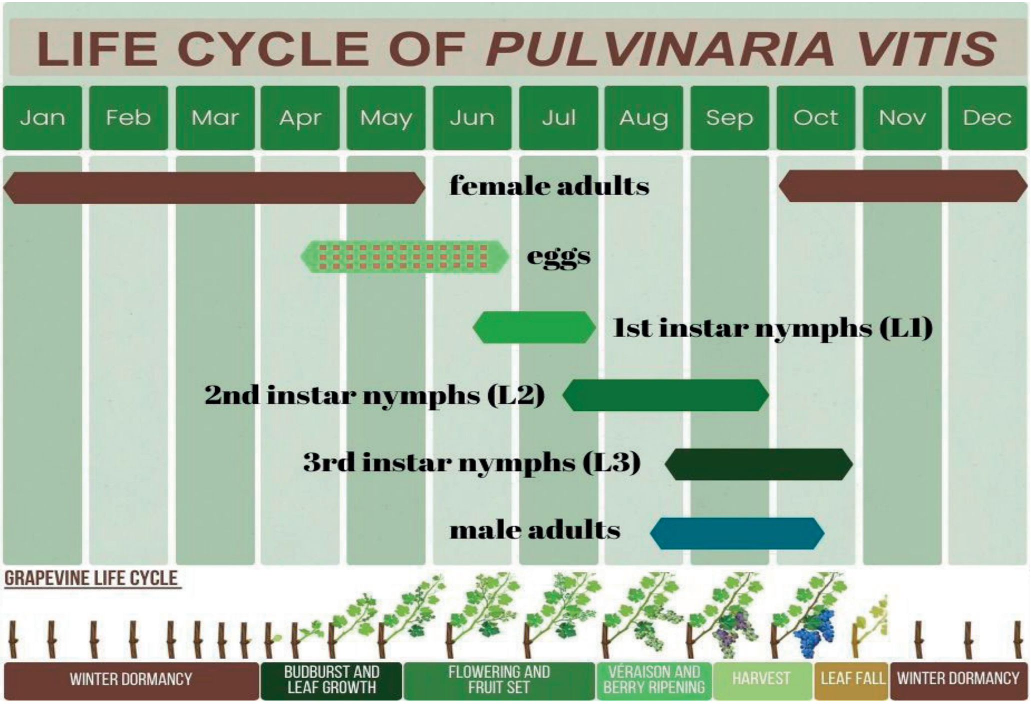

Figure 4. *Pulvinaria vitis* life cycle (adapted after Jansen, 2000) in correlation with the grapevine annual life cycle (described by Venios et al., 2020)

Some important distinctive microscopic characters in differentiating *Pulvinaria vitis* from other Pulvinarias are noted by Milek (2009). This refers to the marginal setae, the long-paired setae from meridian region, the ventral tubular ducts and the dorsal submarginal duct tubercules (Masten Milek et al., 2009).
The biology and ecology of *Pulvinaria vitis* is of great complexity. Future research areas should address the species' adaptability to the recent climate changes, host range expansion, and its interactions with other species within the ecosystem. Even tough significant progress has been made, particularly in the areas of genetic and molecular studies, the continued exploration of *Pulvinaria vitis* remains critical for developing effective management strategies and understanding its ecological impact.

Host range and records

Information on the geographical distribution of scale insect species can act as a reliable indicator of biodiversity across different ecosystems, including natural, agricultural, and urban environments. Such data may also be an indicator on the impacts of climate change over time (Kosztarab & Kozár, 1988).
Originating from the Palaearctic region, *Pulvinaria vitis* has expanded its presence to the Nearctic, Neotropical regions, and New Zealand, showcasing its adaptability and polyphagous nature (Gill, 1988). This scale insect targets a variety of woody plants and has been recognized as a pest affecting especially *Vitis vinifera*, but also other species (Malumphy & John, 2012; García Morales et al., 2016).
In the literature there are some authors documenting its presence on different host species and outbreaks on various parts of the world. J.H.H. Phillips (1962) reported that in Ontario, *Pulvinaria vitis* had a significant economic impact primarily on peach trees (*Prunus persica*). He also noted that laboratory studies showed his ability to infest willow

(*Salix*), poplar (*Populus*), gooseberry (*Ribes grossularia*), fox grape (*Vitis labruscana* Bailey), and hawthorn (*Crataegus* spp.) (Phillips, 1962)

Ben-Dov et al. (2008) reports host plants belonging to the following families: Aceraceae, Betulaceae, Celastraceae, Compositae, Corylaceae, Hippocastanaceae, Juglandaceae, Oleaceae, Rosaceae, Salicaceae, Tamaricaceae, Tiliaceae and Vitaceae (Ben-Dov et al., 2008). It was also recorded in Austria, Vienna by (Kosztarab & Kozár, 1988) on *Corylus* sp. (C. Malumphy & Kahrer, 2011). Acording to C. Malumphy & Kahrer (2011) *Pulvinaria vitis* was recorded feeding on plants belonging to at least 16 families, of which most common on Betulaceae, Grossulariaceae, Rosaceae, Salicaceae and Vitaceae (C. Malumphy & Kahrer, 2011). Also, *Pulvinaria vitis* has been reported as a major pest of *Vitis vinifera* in Croatia, where it was not considered to produce important economic loses until 2006 when a mass outbreak, showed that it can easily become of great economic importance (Masten Milek et al., 2008). The study of Masten Milek et al. (2007), including different grapevine varieties as host plants, found no significant difference in susceptibility between white and red grape varieties, although certain varieties displayed varying levels of sensitivity to infestation (Masten Milek et al., 2007). Additionally, the results of investigation showed that on the same soil type, intensity of infestation can highly variate, so it does not the depend on soil type (Masten Milek et al., 2008).

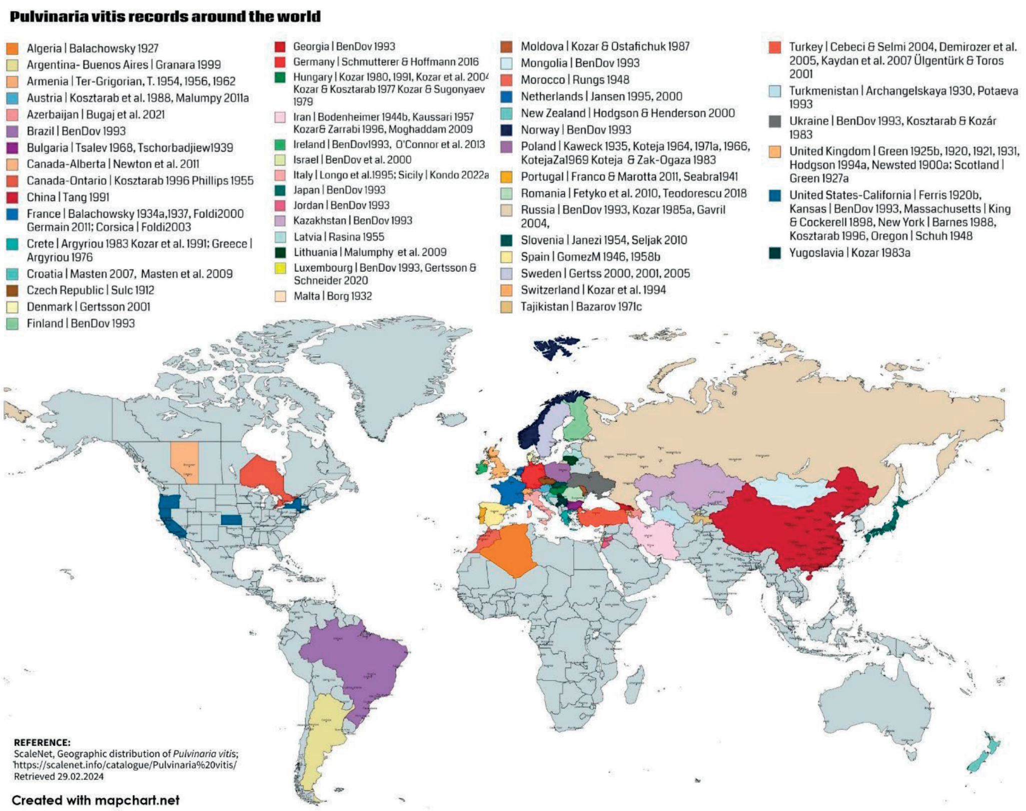

Figure 5. Records of *Pulvinaria vitis* around the world (according to Garcia Morales et al., 2016)

Figure 5 presents a geographic distribution of *Pulvinaria vitis* around the world, based on ScaleNet summarized reports of various authors (Garcia Morales et al., 2016).

The dispersal mechanisms of *Pulvinaria vitis*, particularly in its nymphal stages, are critical for understanding the spread of this pest within and between vineyards. Research has shown that nymphs, once past the crawler stage, can be transported by wind, from infested to uninfested plants (Phillips, 1963). This was evidenced in a controlled insectary setting where third instar nymphs were observed being carried across a six-foot open space, indicating the potential for wind-assisted dispersal in natural environments. Also, the ovisacs of *Pulvinaria vitis* are often found broken, with eggs exposed. These observations suggest that birds, while attempting to feed on the adult scales, may contribute to the spread of *Pulvinaria vitis* eggs by carrying them to other plants (Phillips, 1963). The combination of abiotic and biotic factors highlights the complexity of pest spread in vineyards and the need for comprehensive management strategies that consider multiple vectors of dispersal (Camacho & Chong, 2015).

DAMAGE

Insects can impact the growth and development of plants through direct consumption or indirectly by transmitting viruses and bacteria, or triggering alterations in plant biochemical processes (Zogli et al., 2020; Cooper et al., 2023). Insects equipped with piercing or sucking mouthparts extract nutrients from plants by consuming fluids, through either external digestion of cellular contents or by ingesting from the phloem or xylem (Cooper et al., 2023). This type of feeding can activate plant's immediate defensive mechanisms through the salicylic acid pathway, triggering various indirect defences, including alterations in plant hormone levels or attracting helpful insects by emitting volatile compounds, which could mitigate the impact of these pests (Erb, 2018; Cooper et al., 2023).

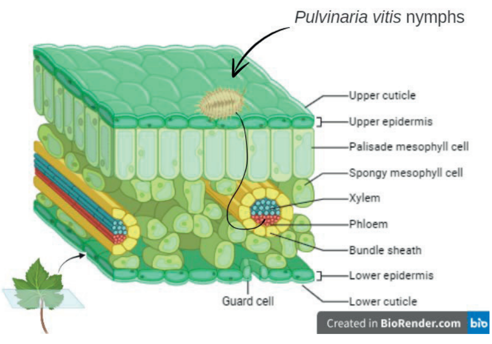

Figure 6. *Pulvinaria vitis* phloem-feeding on leaf

The phloem-feeding behaviour of soft scales, including *Pulvinaria vitis*, on grapevines constitutes a significant area of concern for viticulture, given the consequential impacts on the physiological and biochemical dynamics of the host plants (Hommay et al., 2021). *Pulvinaria vitis,* by its feeding mechanism, taps directly into the phloem sap, a rich source of sugars and other essential nutrients. The stylets are capable of penetrating deep into the plant tissues, reaching the phloem and sometimes even the xylem, which can cause significant damage to the cambium and overall health of the host plant (Phillips, 1963). The saliva of certain species includes proteinases and cellulases, enzymes that can disrupt cells and harm vascular and photosynthetic tissues close to the stylet's penetration point (Camacho & Chong, 2015). While the necrosis caused by single scale insects tends to be localized, extensive damage from severe infestations can result in the dieback of twigs and branches (Vranjic, 1997). This not only weakens the plant but also predisposes it to a spectrum of secondary infections and stress responses. Moreover, the feeding activity of *Pulvinaria vitis* is intricately linked to the transmission of grapevine leafroll-associated viruses. Almeida et al. (2013) emphasized the need for comprehensive research into the biology of leafroll transmission by soft scales, including *Pulvinaria vitis*, which has been identified in Europe as a vector for grapevine leafroll viruses. Their call for further investigation highlights the existing knowledge gap regarding the mechanisms of virus transmission by soft scales (Almeida et al., 2013). Complementing this, Hommay et al. (2021) provides compelling evidence of *Pulvinaria vitis* acting as a vector for Grapevine leafroll-associated viruses -1 and -3, as well as Grapevine virus A, to healthy vine cuttings. This study marks the first documented case of *Pulvinaria vitis* facilitating the grapevine-to-grapevine transmission of these viruses, establishing its significance in the epidemiology of grapevine viral diseases (Hommay et al., 2021).

The phenomenon of sooty mold development on grapevines, as a direct consequence of *Pulvinaria vitis* infestation, represents a significant pathological challenge within viticultural practices. Sooty mold fungi forms on the honeydew excreted during their phloem-feeding activities, forming a black coating on leaf surfaces, stems, and even fruit. Honeydew is comprised of a complex blend of phloem-derived sugars including sucrose, fructose, and glucose, alongside oligosaccharides like erlose and melezitose, which are synthesized by the phytophagous insects (Völkl et al., 1999; Wäckers, 2000). This fungal growth severely impedes the photosynthesis by obstructing light penetration to the leaf surface, thereby compromising the plant's energy acquisition processes. Furthermore, the presence of sooty mold on grape bunches significantly diminishes the aesthetic and market value of the fruit, leading to economic losse. Beyond the direct physical and economic damage, the establishment of sooty mold serves as an indicator of scale insect presence and abundance, suggesting an underlying pest management issue that requires immediate attention (Cooper et al., 2023).

Understanding the specific interactions between *Pulvinaria vitis* and host plants is crucial for developing targeted interventions. Strategies such as the use of resistant grapevine cultivars, the implementation of biological control agents, and the application of systemic insecticides could be optimized based on insights into the feeding behaviour of soft scales. Additionally, monitoring and managing the vector populations at critical points in the grapevine growth cycle could significantly reduce the incidence and spread of leafroll viruses, thereby minimizing the economic and agronomic impacts on viticulture.

MANAGEMENT AND CONTROL

Implementing monitoring strategies is essential for the effective intervention and management of *Pulvinaria vitis* populations in the vineyards. When monitoring *Pulvinaria vitis*, attention should be directed towards identifying partially developed crawlers during pruning activities and locating mature females with their cottony ovisacs in late spring. The presence of honeydew on leaves and fruit in summer also serves as an indicator of infestation (Kabashima & Dreistadt, 2014). This honeydew not only attracts ants but also results in them defending the scales from natural predators as part of a mutualistic relationship. Ant activity, characterized by their rapid movement along vine trunks to access the infested canes, is a sign of scale pest presence (Kabashima & Dreistadt, 2014).

In the modern viticulture context, Integrated Pest Management (IPM) strategies are crucial for ensuring sustainable grape production while reducing the adverse impacts of pest control on the environment. Pertot et al. (2017) highlighted the importance of applying IPM practices in order to promote sustainable pest management approaches (Pertot et al., 2017). IPM strategies integrate biological, cultural, mechanical, and minimal chemical interventions, tailored to the specific ecosystem and pest dynamics of the vineyard.

Biological control relays on natural predators and parasitoids of vineyard pests, reducing the need for chemical pesticides and fostering a balanced ecosystem (Daane et al., 2018). Another example of innovative and effective control practices is provided by Gonçalves et al. (2022), who investigated the application of deep learning technologies on edge devices to automate the identification and quantification of pests using sticky traps in vineyards. Their research demonstrated the effectiveness of the SSD ResNet50 model in accurately identifying a variety of insect species, showing that the deployment of AI-powered methodologies could enhance the efficiency of pest monitoring processes in viticulture. This technology potentially supports the implementation of Integrated Pest Management (IPM) strategies by enabling more timely and informed decision-making (Gonçalves et al., 2022).

Biological control of soft scales, such as *Pulvinaria vitis*, is a key strategy of integrated pest management (IPM) and the framework for sustainable viticulture. Relying on natural enemies to control insect populations is an eco-friendly alternative to the use of chemicals, supporting the goals of protecting the environment and reducing pesticide use. The role of natural predators in the reduction of soft scale populations is accentuated by Rakimov et al. (2015), who advocate for enhancing biodiversity among insect communities as a method of biological control. By fostering a diverse ecosystem of beneficial insects, the reliance on chemical interventions can be reduced, promoting a more sustainable approach to pest management (Rakimov et al., 2015). For example, lady bugs, lacewings, and parasitic wasps are known to target and reduce soft scale numbers effectively (Pertot et al., 2017). Among the natural enemies, parasitoid wasps from the families Aphelinidae and Encyrtidae have been identified as particularly efficacious against soft scale pests, like *Pulvinaria vitis*. These are laying eggs either on or within the soft scales, then the wasp larvae consume the scale insect from within, causing its death (Wang et al., 2019). In a comprehensive study of soft scale parasitism, Abd-Rabou (2011) discovered that *Coccophagus scutellaris* was an efficent parasitic wasp for soft scale insects from Pulvinaria family (Abd-Rabou, 2011). Masten Milek et al. (2009), also reported *Chilocorus bipustulatus* L. and *Exochomus quadripustulatus* L. (fam. Coccinellidae) as predators and *Coccophagus lycimnia* (Walker) (fam. Aphelinidae) as parasitoid of Pulvinaria species (Masten Milek et al., 2009). Paraschiv (2023), studies investigate the efficacy of entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser), *S. feltiae* (Filipjev), and *Heterorhabditis bacteriophora* (Poinar) as biocontrol agents against young females of the Pulvinaria sp. in laboratory settings. These nematodes have shown significant virulence, with *Steinernema carpocapsae*, achieving mortality rates above 90% (Paraschiv, 2023).

Soft scales are occasionally preyed upon by other beetles, hemipterans, thrips, flies, caterpillars, mites, and spiders (Kosztarab, 1996; Rakimov et al., 2015; Camacho & Chong, 2015). Lady bugs (Figure 7) were also reported as soft scale predators, indicating a broader ecological network of possibilities for pest control (Lowery, 2020). Two species of ladybugs (Coccinellidae) *Adalia bipunctata* (L.) and *Exochomus quadripustulatus* (L.), were found to be predators of some scale insects (Paraschiv, 2023). Additionally, bird species such as English sparrows and several warblers, which consume mature soft scales, have been observed to play a crucial role in mitigating infestations (Lowery, 2020).

The mutualistic relationship between soft scales and ants, facilitated by the exchange of honeydew for protection against natural enemies, presents a unique challenge to pests control efforts. Effective ant management, therefore, becomes a critical component of enhancing the efficacy of biological control agents (Kabashima & Dreistadt, 2014).

Figure 7. Ladybugs feeding on *Pulvinaria vitis* nymphs (a, b); ant protecting *Pulvinaria vitis* (c)

Ants have the ability to disrupt their natural competitors by attacking them in order to protect their food source, so if the ants are excluded, there will result an increased population of soft scales specific predators (Camacho & Chong, 2015). Using sugar baits could represent an effective management strategy to distract ants from guarding the scale insects (Beltrà et al., 2017). The use of toxic baits based on boric acid and hydramethylnon has been used for controlling ants, such as *Linepithema micans*, resulting effective in reducing their activity and indirectly controlling scale bugs population (Nondillo et al., 2016).

A key technique for enhancing biodiversity among insect communities in vineyards involves the use of cover crops, which provide habitat and alternative food sources for beneficial insects, increasing their abundance and diversity (Winter et al., 2018). Additionally, planting permanent vegetation such as hedgerows and flower strips within and around vineyards can offer refuge and resources for a variety of insect species, including natural enemies of grapevine pests (Landis et al., 2000). These measures aid beneficial arthropods and enhance the ecological health of the vineyard area. Reducing the usage of broad-spectrum pesticides can help protect non target beneficial insects and promote natural pest management methods (Letourneau et al., 2009). Integrating these approaches, a more resilient agroecosystem that supports a rich community of beneficial insects, enhancing both pest control and pollination, can be created (Tomoiagă, 2022).

While the focus shifts towards reducing dependence on chemical pesticides, their use remains a necessary part of an IPM. Selective insecticides that minimize harm to non-target species and the environment, including oil-based products, have shown efficacy in managing soft scale infestations on grapevines (Mansour et al., 2017). When using chemical treatments, it is essential to follow label instructions and consider the potential impact on non-target species, including beneficial insects and pollinators, as well as the potential for resistance development in the pest population. It is important to note that the timing of insecticide application is crucial for maximizing efficacy and minimizing the impact on beneficial insects, also treatments are usually more effective when applied early in the season, targeting the crawler stage before they settle and form protective waxy coatings (Lowery, 2020).

Phillips et al. reported in 1962 an example of chemical treatment that disrupted the natural biological control of *Pulvinaria vitis* and lead to serious outbreaks. The widespread application of DDT, which frequently was used in an insufficient quantity to kill the scale pest, but adequate to kill its natural parasites, exacerbated and prolonged the outbreak from Niagara Peninsula in 1945 (Phillips et al., 1962).

The resistance of grapevine pests to chemical treatments is an increasingly complex issue, with studies showing varying effectiveness of both chemical and natural resistance inducers. To understand the resistance of grapevine pests, specifically soft scales, to insecticides, the study of Quesada et al. (2018) is offering valuable insights that could aid in the development of efficient strategies for managing soft scales. His study highlights the biological variability among scale insect species and how it can lead to differences in susceptibility to various insecticides. The research found that bifenthrin and pyriproxyfen were the most effective insecticides for soft scale insects. The article also discusses how the duration of crawler activity can decrease the efficacy of insecticides, emphasizing the importance of timing in the application of treatments (Quesada et al., 2018). Systemic insecticides successfully control specific scale insect species with a single annual application (Camacho & Chong, 2015). The application is usually done right before the crawler emerges to maximize the concentration of active chemicals in the plant tissues.

Neonicotinoids, although offering advantages like flexibility and long-lasting effects, should be cautiously used due to their potential harm to pollinator health and their involvement in spider mite outbreaks (Camacho, 2015).

Targeting crawlers and settled first instars, which lack or have only a thin protective wax layer, may result in a greater efficacy, given that the body of older nymphs and adults with soft scales is covered in a layer of wax that is impenetrable to aqueous insecticide solutions (Kosztarab, 1996; Marotta, 1997; Camacho & Chong, 2015).

Research conducted by Wallingford et al. (2015) demonstrates the effectiveness of insecticide applications, specifically acetamiprid and spirotetramat, in controlling the vector insects from the Coccidae family. It has been demonstrated that this insecticide significantly reduces populations of *Pseudococcus maritimus,* a mealybug species, in vineyards (Wallingford et al., 2015). While not directly related to *Pulvinaria vitis*, the effectiveness of spirotetramat on mealybugs suggests it could be effective for controlling soft scale insects as well, given their similar habits(Wallingford et al., 2015).

For the management of the crawler stage in Pulvinaria infestations, malathion and insecticidal soap have been identified as effective treatments (Lowery, 2020). Continuous monitoring and potential repeat applications are necessary as long as crawler activity is detected. Furthermore, Movento™ (spirotetramat) has been approved for use on grapevine within the growing season specifically for soft scale control, although its application on table grapes is not advised (Lowery, 2020).

An alternative for chemical treatments could be the use of botanical extracts and essential oils. However, more comprehensive studies including field application and phytotoxicity tests are needed to fully realize the potential of botanical compounds in controlling grapevine soft scales and other pests (Peschiutta et al., 2018). Additionally, monitoring or mating disruption of soft scales with pheromone baits could represent a solution for *Pulvinaria vitis* population control, but this method is not available at the moment (Camacho & Chong, 2015).

The cultural control is also important in IPM by making the environment less favourable to pest development and reproduction. Proper fertilization, pruning, and irrigation maintain plant vigour, promote plant tolerance to pest damage, and reduce sap-sucking insect population growth (Kabashima & Dreistadt, 2014). However, few studies have demonstrated the efficacy and underlying mechanism of these cultural management practices (Camacho & Chong, 2015). IPM strategies should take in account that, modifying vineyard management and soil conservation practices can indirectly influence soft scale dynamics by affecting grapevine health and resilience. Techniques such as cover cropping, mulching, and the application of organic amendments have been shown to improve soil health and grapevine vigour, potentially reducing the susceptibility to pest infestations (Cataldo et al., 2021; Payen et al., 2021). Camacho, 2015 stated that nutrients in the soil and the plant also affect the severity of scale insect infestation. It has been demonstrated that plants provided with more nitrogen, potassium, and organic compost amendments could support more severe scale infestations, than poorly fertilized plants (Camacho, 2015).

Improvement and adaptation of IPM strategies might include adopting proven technologies from other areas or exploring new methods of pest control, such as the use of natural substances (Santos et al., 2020), AI-powered technologies and pheromone mating disruption. The effective management of these pests is a dynamic process, requiring constant vigilance, innovation, and the ability to adapt, as both pests and diseases can evolve in response to the environment and the control measures implemented by vinegrowers (Santos et al., 2020).

CLIMATE CHANGE INFLUENCE

Global climate change, marked by increasing temperatures and shifting precipitation patterns, is expected to intensify over the coming decades. These changes are anticipated to alter the geographic distribution of grapevine varieties and rootstocks, as temperature plays a central role in determining viable cultivation areas, phenophases, the yield and quality of grape production, diseases and grapevine pest distribution (Colibaba et al., 2024). By influencing the phenology and pest dynamics, the context of climate change imposes revised pest management strategies to address the increased pest pressure on grapevine crops.

Temperature records in viticultural areas reveal that during the growing seasons from 1950 to 2000 the mean temperature has increased by about 1.6-1.8°C in Europe and 1.2-1.4°C worldwide (Venios et al., 2020). Changes in grapevine growth and physiological development have been well documented in correlation with rising temperatures in recent decades (Jones & Davis, 2000; Duchêne et al., 2010; Santos et al., 2020).

In light of anticipated changes in climate, wine-producing regions may face heightened pest and disease threats, necessitating increased efforts in plant protection at a time when reducing the environmental impact of such treatments is crucial. A detailed simulation study, as reported by Santos et al. (2020), investigating the potential variations in insect pest resilience according to geographical latitude, found a decrease in resilience near the equator and an increase resilience in regions located beyond the 30° latitude lines (Santos et al., 2020). Observations over the past thirty years regarding pests affecting grapevines have revealed changes in the timing of grape berry moth activities, alterations in the geographic distribution of leafhoppers that transmit grapevine diseases, and an expansion in the habitat of grapevine mealybugs (Santos et al., 2020).

Scale insects, including *Pulvinaria vitis*, presents a high degree of sensitivity to temperature fluctuations due to their poikilothermic nature (Skendžić et al., 2021). This sensitivity has significant implications for their metabolic rates, reproductive capacity, survival, and dispersal capabilities, potentially affecting their geographic range and population dynamics. As noted by Skendžić et al. (2021), such changes could result in increased pest pressures across new areas and a wider range of host plants (Skendžić et al., 2021). Robinet & Roques (2010) further elucidate that rising temperatures may enhance winter survival and accelerate development rates, leading to a faster progression through life stages and possibly more generations per year. Instead, elevated temperatures could increase metabolic rates without a corresponding rise in fertility or survival, indicating a complex relationship between temperature and insect physiology. Climate change could allow *Pulvinaria vitis* to colonize previously unsuitable regions due to warmer conditions, highlighting the complex impact of climate change on the distribution and life history of this grapevine pest (Robinet & Roques, 2010; Skendžić et al., 2021).

Climate change can lead to earlier onset of phenological stages in grapevines, potentially altering the synchrony between grapevines and the lifecycle of pests like *Pulvinaria vitis* (Reineke & Thiéry, 2016). Warmer temperatures may speed up the development cycles of both grapevines and pests, but not necessarily in synchrony. This could affect the timing and intensity of pest infestations, complicating existing management strategies (Reineke & Thiéry, 2016). Furthermore, elevated CO_2 levels, higher temperatures, and changing precipitation regimes can directly impact pest populations by extending their geographic range into areas previously unsuitable due to climatic conditions. This includes the potential for increased *Pulvinaria vitis* activity in cooler viticultural regions that become warmer and more hospitable to pests under climate change scenarios (Blanco-Ward et al., 2021; Castex et al., 2023). One potential consequence of climate change-induced temperature increases is the facilitation of more pest generations per growing season. Furthermore, this could be compensated by the anticipation of earlier fruit maturation and harvest dates, which would restrict the harmful effects of late-season pest generations (Caffarra et al., 2012). Shifts in tritrophic relations due to climate change are also concerning for Integrated Pest Management (IPM), as natural enemies of pests may suffer from warmer temperatures, affecting their synchronization with pest populations. Understanding these changes at various levels and their linkages is crucial for adapting IPM strategies to future climatic conditions (Castex et al., 2018). Continues research and monitoring are essential to understand the evolving relationship between grapevine and pests like *Pulvinaria vitis* in the context of global warming, ensuring the sustainability of viticulture in a changing climate (Biasi et al., 2019; Comșa et al., 2022). The adaptive practices should be introducing the use of resistant grapevine varieties, adapting the management strategies to cope with heat and water stress, and implementing innovative pest and disease management scheme in order to reduce the use of pesticides (Pertot et al., 2017). A comprehensive 21-year study conducted in the southern region of Romania has provided evidence of significant climatic changes impacting viticulture, specifically grape yield and quality (Zaldea et al., 2021). This research underscores the regional implications of climate change on viticulture. Further investigations into the climatic conditions affecting viticulture in Romania have highlighted a trend towards higher minimum temperatures during winter, alongside considerable variability in thermal conditions. Such alterations in key viticultural climatic indices, including the Huglin and Winkler indices, point to a transition towards warmer growing seasons, which could lead to mass outbreaks of scales pests on grapevine (Bucur et al., 2019; Zaldea et al., 2021).

Similar climate changes have also been reported in Târnave vineyard by (Răcoare et al., 2022) and (Chedea et al., 2021). Târnave vineyard is the most prestigious and appreciated viticultural area of Transylvania and it is located in the central-north-vest region of Romania. Between 2000 and 2020, compared to the reference period of 1975-2007 the Târnave vineyard experienced a significant climatic shift, with the average annual temperature rising by 1-1.5°C; annual useful temperatures increased by more than 200°C; the length of the active vegetation period has increased by approx. 15-20 days and the frost periods (late spring and early autumn frosts) have reduced considerable (Chedea et al., 2021). These environmental changes, may have facilitated faster development cycles and higher survival rates of *Pulvinaria vitis*, aligning with the species' thermal preferences and may also have disrupted the phenological synchrony between grapevines and their pests easing the expansion of *Pulvinaria vitis* populations. In the last few years, more and more wine growers from this area have signalled the presence of *Pulvinaria vitis* outbreaks and they have serious concerns regarding the economic impact on the grape production. We are currently collecting data and investigating this matter.

Within the framework of ongoing climate change, coupled with the intensification of global trade, it is projected that pest species such as *Pulvinaria vitis* will experience outbreaks and extend their presence to new regions within Romania. The anticipated warmer climate conditions are likely to enhance the survival prospects of this pest, facilitating its expansion towards northern latitudes. Furthermore, the expected increase in temperatures, particularly in areas surrounding human settlements, may lead to a higher presence of such pests in these areas in the near future (Paraschiv, 2023).

CONCLUSIONS

This review summarizes the state of knowledge regarding the biological aspects and management approaches of the grapevine soft scale pest, *Pulvinaria vitis*, within the context of climate change, while identifying future research needs and gaps.

Having a polyphagous nature and presenting morphological variability depending on the host plant, or environmental conditions, *Pulvinaria vitis* was sometimes confused with other soft scale species. *Pulvinaria vitis* can be hard to identify, especially in the nymphal stages. The adult females can be identified by the cottony ovisacs and the distinctive features of the body: 3-8 mm long, ovoid to circular, dark brown, presenting 3-5 transversal striae.

The phloem-feeding behaviour of *Pulvinaria vitis* impacts the growth and development of the grapevine through direct consumption, but also, it could represent a vector for Grapevine leafroll-associated viruses.

The phenomenon of sooty mold development on the honeydew excreted during their phloem-feeding activities, also represents a significant pathological challenge within viticultural practices. This fungal growth restricts the photosynthesis by obstructing light penetration to the leaf surface and also the respiration and transpiration process.

It has been estimated that the scale insects produce agricultural losses that can globally surpass 1 billion dollars per year (Kozár et al. 2009). In Romania, there are no recent studies regarding the economic impact of *Pulvinaria vitis* on viticulture although in the last few years more and more outbreaks are reported by winegrowers.

Scale insects, including *Pulvinaria vitis*, present a high degree of sensitivity to temperature fluctuations. Recent climate changes consist in temperature rises and precipitation pattern shifts which can accelerate *Pulvinaria vitis* life cycle, increase their winter survival rates, and expand their geographic ranges, potentially leading to more frequent and severe outbreaks.

In order to manage these challenges, the implementation of Integrated Pest Management (IPM) strategies, which include biological, cultural, mechanical, and minimal chemical interventions, is crucial for a sustainable grape production while minimizing environmental impacts. Techniques such as cover cropping, mulching, and applying organic amendments can enhance soil health and grapevine vigour, reducing susceptibility to pest infestations. Adjustments in pesticide application timing are also important in order to reduce the impact on useful fauna and to avoid developing resistance. The evolution of climate change and local conditions must be considered while implementing these strategies.

Continue research in a multidisciplinary approach is essential in the process of elaborating effective IPM strategies to face the future climatic conditions. This could be achieved by integrating knowledge on pest biology, transmission of viruses, targeted insecticide applications, sustainable pest control techniques.

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EVALUATING THE APPLICABILITY OF PASSIVE REMOTE SENSING TECHNOLOGY WITH A MULTIROTOR DRONE IN PRECISION VITICULTURE

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Abstract

In recent years, there has been a notable increase in the use of Unmanned Aerial Vehicles (UAVs) for precision viticulture. This study investigates the practical applications of passive remote sensing with drones in vineyards. Three distinct winegrowing regions in Romania were chosen, each characterized by varying grape varieties and climatic conditions. Utilizing a semiprofessional drone equipped with a high-resolution multispectral sensor, the study aimed to rapidly identify missing vines, generate parcel-level elevation maps, and validate the correlation of the NDVI vegetation index with vine vigour. The study unveiled significant results, covering plant counting reports, elevation maps detailing the exposition and inclination of various parcels, and NDVI vigor maps. In summary, this research contributes to the progression of precision agriculture in viticulture through the application of remote sensing and vegetation indices. The implications of the findings and potential applications are discussed, with a SWOT analysis providing insights into future prospects.

Key words: *precision viticulture, UAV, elevation map, missing vines, NDVI vigor map.*

INTRODUCTION

Remote sensing is the branch of geomatics that deals with the detection, measurement, and recording of electromagnetic radiation reflected or transmitted by an object, area, or phenomenon without the sensor being in direct contact with the subject being analyzed. Remote sensing relies on recording images with color differences, and its main objectives are the identification and evaluation of Earth components along with their physico-chemical properties. In the case of passive remote sensing, reflected solar radiation is primarily measured in the visible, near-infrared, and mid-infrared spectra. In the visible and infrared spectrum, the measured energy is influenced by properties such as pigmentation, moisture, and cellular structure in the case of vegetation, mineralogical composition and moisture in the case of soil, and sedimentation level in the case of water. The specific spectral signatures are exemplified in Figure 1 (Portengen, 2017). Due to the potential for the rapid delivery of comprehensive maps regarding the shape, size, and vigor of the vineyard, passive remote sensing represents a powerful tool in precision viticulture. The interpretation of multispectral images can efficiently support the optimization of winery activities by facilitating optimal grapevine management decisions. "Remote sensing applications include mapping and monitoring soil properties, grapevine variety classification, pest management, plant water stress detection, analysis of leaf chemical content, and weed control monitoring." (Zhang, 2012).

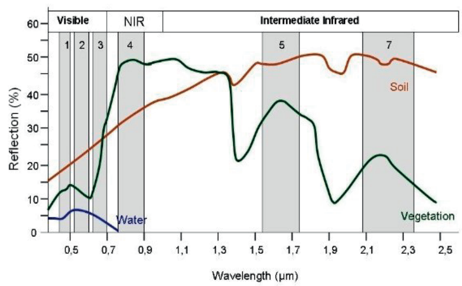

Figure 1. Typical spectral signatures for vegetation, water, and soil

Over 150 vegetation indices have been documented in scientific literature; however, only a subset undergo systematic testing and practical application. The most widely utilized index is the Normalized Difference Vegetation Index (NDVI). Developed by NASA in the 1970s, NDVI represents the nonlinear transformation of the red and near-infrared (NIR) bands, calculated as the difference between these two bands divided by their sum (Nerțan A., 2016):

$$\mathrm{NDVI} = \frac{(\mathrm{NIR}-\mathrm{RED})}{(\mathrm{NIR}+\mathrm{RED})}.$$

The underlying concept of this index is that plants reflect near-infrared light to prevent drying and absorb red light for photosynthesis. NDVI is recognized as a good measure of vegetation development and density, associated with biophysical parameters such as biomass, leaf area index, vegetation cover percentage, and vegetation photosynthetic activity. "Parcel separation can be successfully achieved using NDVI, correlated with soil electrical conductivity and the number of clusters" (Urretavizcaya 2017). The optimal period for obtaining NDVI for this analysis is after flowering and until veraison. "NDVI is directly correlated with the amount of active photosynthetic radiation a plant can absorb." (Kavak et al., 2014).

The overall objective of this work is to evaluate the applicability of remote sensing technology in vine cultivation through the analysis of digital maps. This idea further branches into three specific objectives. In the first phase, elevation maps will be generated to understand more precisely the geographical characteristics of mesoclimates, such as altitude, slope, exposure, and even soil water reserve flow. The second objective is to assess gaps in the plantation, missing grapevines that lead to a waste of phyto-sanitary substances and a decrease in yield. The third objective, the most complex one, involves correlating canopy vigour with the NDVI vegetation index map.

MATERIALS AND METHODS

For the research a semi-professional multirotor drone DJI Phantom 4 Advanced was used, equipped with a 25.4 mm CMOS sensor capable of recording 4K video and 20-megapixel photos and a multispectral camera, Sentera Double 4K NDVI/NDRE fitted with a BSI CMOS - Sony Exmor R™ IMX377 sensors having a resolution of 12.3 megapixels. The software programs used to generate content were Pixel4D Fields for elevation maps, Agremo for plant counting reports, with trial subscriptions, and Field Agent from Sentera for NDVI map, with monthly subscription. All flight campaigns took place on clear sky conditions at noon when the sunlight falls perpendicularly on the canopy to minimize shadows between rows.

The research was conducted throughout the 2020 vintage in several viticultural areas: Marcea Winery (Marcea) – DOC Stefanesti, Pietroasa-Istrita Research and Development for Viticulture and Pomiculture Didactic Station (Pietroasa)– DOC Pietroasa, and Girboiu Winery (Girboiu) – DOC Cotesti. At Marcea two plots of Feteasca Neagra were analysed one with a surface of 2.3 hectares, shortly named as FN1, and the second one with a surface of 2.4 hectares briefly noted as FN2. Distance between plants was 1 meter and row spacing 2.1 meters, resulting in a planting density of 4,545 plants per hectare. In plot FN1, the row orientation was North-South, while for plot FN2, the row orientation was Northwest-Southeast. At Pietroasa, the study was conducted on two plots, first planted with Italian Riesling on a surface of 2.5 hectares, briefly noted as IR. The second plot planted with Cabernet Sauvignon on a surface of 2.46 hectares, shortly named CS. For both plots the distance between plants was 1.2 meters and 2 meters between rows, with rows orientation North-East – South-West. At Gîrboiu Winery, the research took place on one plot of Sauvignon Blanc located near the winery with a surface of 6.3 hectares, referred to as SB. The space between plants was 1 meter and 2.3 meters between rows, resulting in a planting density of 4670 vines per hectare. The row orientation was North - South.

For each plot, various research studies were conducted, as presented in Table 1.

The control method for NDVI vigour map was done by correlation with two vine measurements: the Leaf Wall Area (LWA) and the Leaf Row Volume (LRV) obtained by calculating the height and width of the canopy according to the formulas:

$$Leaf\ Wall\ Are = Canopy\ Height\ \times 2\ \times 10000$$

(where 2 is the number of faces of a row and 10000 is the area of a hectare expressed in meters).

$$Leaf\ Row\ Volume = Canopy\ Height\ \times Canopy\ Width \times 10000$$

(where 10000 is the area of a hectare expressed in meters).

All measurements were taken using a retractable tape measure. For each parcel a different number of vines were sampled to ensure a better representativity cover of the entier variability. In FN2 parcel were measured 37 vines, in IR parcel were measured 44 vines and in SB1 parcel were measured 77 vines.

Table 1. Research done by parcel and phenological stage

Winery	Parcel	Date	Phenological stage	Research
Marcea	FN 1	11.May	Before flowering	Elevation map
Marcea	FN 2	11.May	Before flowering	Elevation map
Marcea	FN 2	2.July	After flowering	NDVI map
Pietroasa	IR	9.July	After flowering	NDVI map
Pietroasa	CS	19.May	Before flowering	Elevation map
Pietroasa	CS	19.May	Before flowering	Missing plants
Gîrboiu	SB	8.May	Before flowering	Elevation map
Gîrboiu	SB	8.May	Before flowering	Missing plants
Gîrboiu	SB	6.June	Flowering	NDVI map

RESULTS AND DISCUSSIONS

At Marcea Winery the altitude detected in both parcels was high, over 400 meters, which could be an advantage in the context of global warming. If in the past, the area was suitable for the production of white wines, but the potential for red wines has increased in last decades. The exposition of parcel FN1 is North West (Figure 2), and the exposition of parcel FN2 is South East (Figure 3). A very warm year favors the ripening of the grapes in parcel FN1, while a normal or cooler year favors the ripening of grapes in parcel FN2. The slope of parcel FN1 is medium to high, being from 5% to 18%. Mechanized work in the northern part of the parcel being almost impossible. The slope of parcel FN2 is gentle to moderate, being 8% and 10%. Except for the northern zone of parcel FN1 where the slope is high, the locations of the parcels are very good.

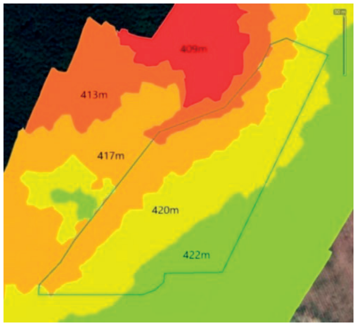

Figure 2. FN1 elevation map

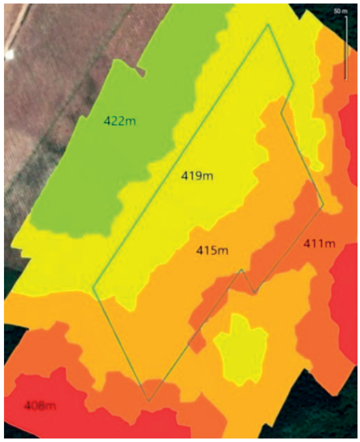

Figure 3. FN2 elevation map

At Pietroasa the altitude detected in CS parcel was around 100 meters with an exposition to South (Figure 4), benefiting uniformly from the maximum number of hours of solar radiation, and a slope of approximately 9%, representing a gentle to moderate slope.

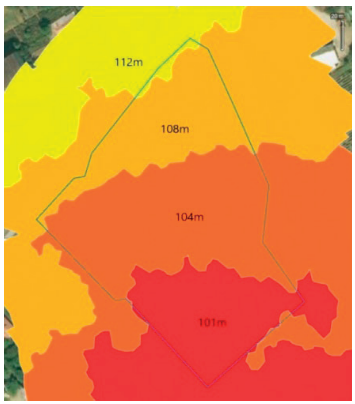

Figure 4. CS elevation map

At Girboiu the average altitude found was around 170 meters, representing a medium altitude for Romania, in the context of grape growing. The exposition of the SB parcel is East (Figure 5), benefiting from rapid morning warming and a decrease in solar radiation in the afternoon. The slope of the SB parcel is approximately 3%. Having a small slope the location of the parcel allows easy mechanized work of the vineyard, with the strong point being the North-South orientation of the rows.

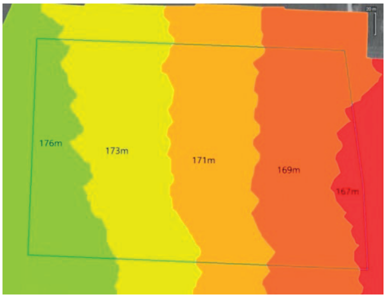

Figure 5. SB1 elevation map

Plant count report is crucial for obtaining a more accurate understanding of gaps in the plantation and helps optimize the cost of planting vine material acquisition. Using a drone for such calculations could be time effective. For the CS parcel at Pietroasa, the calculations resulted in approximately 8,340 plants, 1,908 plants less than the potential for the plot. The land occupancy rate is 19% below the norm, indicating a need to replant 1,908 vines (Figure 6).

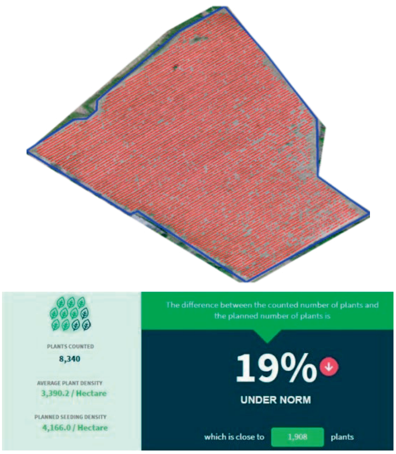

Figure 6. CS plant count report & map

At Girboiu, for the SB parcel, approximately 25,305 plants were calculated, with 2,168 plants less than the potential for the land.
The land occupancy rate is 8% below the norm, indicating a need to replant 2,168 vines (Figure 7).

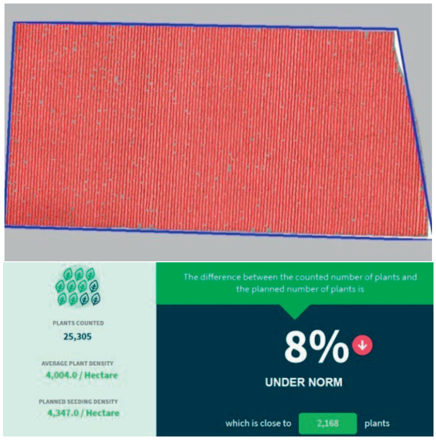

Figure 7. SB plant count report & map

NDVI vigor map. To demonstrate, correlation values were calculated for the LWA and LRV, which were subsequently associated percentage-wise with the colours resulting from the processing of the NDVI maps.

Table 2. FN2 canopy measurements

	Vine 8	17	24	34	40	51	56	68
Row 9	2/0.5		2.2/0.5					
18		2.2/0.5		2.2/0.4		2.2/0.45		2.2/0.4
27	2.3/0.4		2.6/0.4		2.2/0.4		2.5/0.4	
36		2.5/0.4		2.5/0.5		2.4/0.55		2.6/0.4
45	2/0.6		2.4/0.4		2.95/0.4		2.15/0.5	
54		2.7/0.6		2.6/0.6		2.5/0.5		
63	2.2/0.6		2.2/0.55		2.1/0.5		2.3/0.6	
72		2.4/0.5		2.5/0.4		2.1/0.35		2.6/0.4
81	2.2/0.4		2.2/0.5		2.1/0.5		2.3/0.5	
90		2.5/0.4		2.65/0.5		2.5/0.5		2.3/0.4

For parcel FN2 at Marcea Winery, canopy height and width measurements are recorded in Table 2, organized by rows and vine number. Table 3 contains LWA values and Table 4 contains LRV values. The values in Tables 3 and 4 were correlated with the vegetation index NDVI map shown in Figure 8, and small differences were found-below 0.12% for both LWA and LRV, as indicated by the calculations in Table 5.

Table 3. FN2 LWA calculations

	Vine 8	17	24	34	40	51	56	68
Row 9	19047		20952					
18		20952		20952		20952		20952
27	21904		24761		22857		23809	
36		23809		23809		22857		24761
45	19047		21904		28095		20476	
54		25714		24761		23809		
63	20952		20952		20000		21904	
72		22857		23809		20000		24761
81	22857		20952		20000		21904	
90		23809		25238		23809		21904

Table 4. FN2 LRV calculations

	Vine 8	17	24	34	40	51	56	68
Row 9	4761		5238					
18		5238		4190		4714		4190
27	4380		4952		4571		4761	
36		4761		5952		6285		4952
45	5714		4380		5619		5119	
54		7714		7428		5952		
63	6285		5761		5000		6571	
72		5714		4761		3500		4952
81	4571		5238		5000		5476	
90		4761		6309		5952		4380

The orange and red colors in the tables and NDVI map correspond to LWA values ranging from 19,047 to 20,952 m²/ha and LRV values between 3,500 and 4,761 m³/ha, representing approximately 38% of the analyzed area for the FN2 plot. The dark green and light green colors correspond to LWA values ranging from 21,904 to 28,095 m²/ha and LRV values ranging from 4,952 to 7,714 cubic m³/ha, representing approximately 62% of the analyzed area.

Figure 8. FN2 NDVI vigor map

Table 5. FN2 NDVI & LWA-LRV correlation

	NDVI map		
Colour	Value	Surface (ha)	Percent
Green	0.26-0.92	1,42	62.28%
Orange	0.19-0.25	0.86	37.72%
		2.28	
	Leaf Wall Area		
	Number of vines	Interval (m²/ha)	Percent
	14	21904-28095	62.16%
	23	19047-20952	37.84%
	37		
	Leaf Row Volume		
	Number of vines	Interval (m³/ha)	Percent
	14	4952-7714	62.16%
	23	3500-4761	37.84%
	37		

For the IR plot at Pietroasa, height and width measurements in meters are recorded in Table 6 by rows and vines. LWA values are found in Table 7, LRV values in Table 8, and the NDVI map is shown in Figure 9. The values from Tables 7 and 8 were correlated with the NDVI map, resulting in differences below 3.7% for LWA and below 0.3% for LRV, as indicated by the analysis in Table 9.

Table 6. IR canopy measurements

	Vine 9	23	35	45	60	80	95	110
Row 8	1.35/ 0.45	1.35/ 0.55	1.4/ 0.5	1.45/ 0.5				
16	1.35/ 0.45	1.25/ 0.5	1.35/ 0.55	1.3/ 0.5	1.15/ 0.5			
24	1.4/ 0.6	1.45/ 0.5	1.2/ 0.5	1.35/ 0.4	1.2/ 0.5			
32	1.35/ 0.45	1.3/ 0.45	1.45/ 0.45	1.2/ 0.45	1.45/ 0.45	1.4/ 0.4		
48	1.3/ 0.45	1.4/ 0.45	1.45/ 0.5	1.2/ 0.45	1.15/ 0.4	1.25/ 0.4	1.2/ 0.45	1.5/ 0.5
64	1.1/ 0.4	1.15/ 0.4	1.1/ 0.35	1.15/ 0.4	1.3/ 0.35	1.2/ 0.45	1.4/ 0.45	1.25/ 0.4
72	1.15/ 0.35	1.05 /0.3	1.1/ 0.45	1.15/ 0.3	1.1/ 0.45	1.3/ 0.4	1.25/ 0.45	1.1/ 0.35

Table 7. IR LWA calculations

	Vine 9	23	35	45	60	80	95	110
Row 8	13500	13500	14000	14500				
16	13500	12500	13500	13000	11500			
24	14000	12500	13500	13000	12000			
32	13500	13000	14500	12000	14500	14000		
48	13000	14000	14500	13000	11500	12500	12000	15000
64	11000	11500	11000	11500	13000	12000	14000	12500
72	11500	10500	11000	11500	11000	13000	13000	11000

Table 8. IR LRV calculations

	Vine 9	23	35	45	60	80	95	110
Row 8	3000	3700	3500	3600				
16	3000	3100	3700	3250	2800			
24	4200	3600	3000	2700	3000			
32	3000	2900	3250	2700	3250	2800		
48	2900	3150	3600	3250	2300	2500	2700	3750
64	2200	2300	1900	2300	2300	2700	3150	2500
72	2000	1500	2400	1700	2500	2600	2800	1900

Figure 9. IR NDVI vigor map

Interpretation of the tables and NDVI map for the IR plot in Pietroasa: The red color represents approximately 32% of the total plot and corresponds to LWA values ranging from 10,500 to 11,500 m²/ha and LRV values between 1,500 and 2,500 m³/ha. The yellow color represents approximately 34% of the total plot and corresponds to LWA values ranging from 12,000 to 13,000 m²/ha and LRV values between 2,600 and 3,000 m³/ha. The green color represents approximately 34% of the total analyzed area and corresponds to LWA values ranging from 13,500 to 14,500 m²/ha and LRV values between 3,500 and 4,200 m³/ha

Table 9. IR NDVI & LWA-LRV correlation

	NDVI map		
Colour	Value	Surface (ha)	Percent
Green	0.26-0.45	0.81	34%
Yellow	0.20-0.25	0.82	34.3%
Red	0.11-0.20	0.76	31.7%
		2.39	
	Leaf Wall Area		
	Number of vines	Interval (m²/ha)	Percent
	16	13500-14500	36%
	16	12000-13000	36%
	12	10500-11500	28%
	44		
	Leaf Row Volume		
	Number of vines	Interval (m³/ha)	Percent
	16	3100-4200	34%
	16	2600-3000	34%
	12	1500-2500	32%
	44		

For the SB plot at Girboiu, height and width measurements in meters are recorded in Table 10. LWA values are found in Table 11, VRF values in Table 12, and the NDVI vigor map is depicted in Figure 10. All three tables were correlated, with differences below 3.26% for LWA and below 1.96% for VRF, as indicated by the analysis in Table 13.

Table 10. SB canopy measurments

	Vine 18	22	53	65	88	106	122	148	159	190	194
Row 5	0,9/ 0.7		1/ 1		0.8/ 0.7		0.9/ 0.9		1/ 0.9		1/ 0.7
14		0.9/ 0.8		1.2/ 0.75		1.1/ 1.05		1.2/ 0.85		0.9/ 1.1	
23	0.75/ 0.75		1/ 0.75		1/ 0.4		1/ 0.6		0.7/ 0.4		0.8/ 0.5
32		1/ 0.6		1.1/ 0.6		0.75/ 0.65		1.05/ 0.65		0.9/ 0.8	
41	0.9/ 0.6		0.75/ 0.55		0.9/ 0.7		0.9/ 0.6		0.9/ 0.65		0.5/ 0.4
50		0.6/ 0.5		0.5/ 0.4		0.9/ 0.7		0.7/ 0.5		0.75/ 0.6	
59	0.8/ 0.7		0.9/ 0.5		0.9/ 0.5		0.9/ 0.7		0.8/ 0.5		0.7/ 0.5
68		0.7/ 0.7		0.6/ 0.5		0.7/ 0.6		0.9/ 0.7		0.8/ 0.65	
77	0.85/ 0.7		0.7/ 0.6		0.9/ 0.6		0.8/ 0.6		0.7/ 0.6		0.8/ 0.5
86		0.6/ 0.5		0.7/ 0.6		0.8/ 0.7		0.75/ 0.6		0.8/ 0.6	
95	0.7/ 0.5		0.6/ 0.6		0.6/ 0.4		0.6/ 0.6		0.7/ 0.65		0.8/ 0.6
104		0.85/ 0.5		0.9/ 0.7		0.6/ 0.5		0.9/ 0.6		0.9/ 0.6	
113	0.9 /0.6		0.6/ 0.5		0.5/ 0.4		0.9/ 0.6		0.65/ 0.6		0.8/ 0.5
122		0.9/ 0.55		0.7/ 0.5		0.9/ 0.6		0.8/ 0.5		0.7/ 0.6	

Table 11. SB LWA calculations

	Vine 18	22	53	65	88	106	122	148	159	190	194
Row 5	2739		4347		2434		3521		3913		3043
14		3130		3913		5021		4434		4304	
23	2445		3260		1739		2608		1217		1739
32		2608		2869		2119		2967		3130	
41	2347		1793		2739		2347		2543		869
50		1304		869		2739		1521		1956	
59	2434		1956		1956		2739		1739		1521
68		2130		1304		1826		2739		2260	
77	2586		1826		2347		2086		1826		1739
86		1304		1826		2434		1956		2086	
95	1521		1565		1304		1565		1978		2086
104		1847		2739		1304		2347		2347	
113	2347		1304		869		2347		1695		1739
122		2152		1521		2347		1739		1826	

Table 12. SB LRV calculations

	Vine 18	22	53	65	88	106	122	148	159	190	194
Row 5	7826		8695		6956		7826		8695		8695
14		7826		10434		9565		10434		7826	
23	6521		8695		8695		8695		6086		6956
32		8695		9565		6521		9130		7826	
41	7826		6521		7826		7826		7826		4347
50		5217		4347		7826		4347		6521	
59	6956		7826		7826		7826		6956		6086
68		6086		5217		6086		7826		6956	
77	7391		6986		7826		6086		6086		6956
86		5217		6086		6956		6521		6956	
95	6086		5217		5217		5217		6086		6956
104		7391		7826		5127		7826		7826	
113	7826		5217		4347		7826		5652		6956
122		7826		6086		7826		6956		6086	

Interpretation of the tables and NDVI map for the SB plot at Girboiu:
The orange and red colors correspond to LWA values ranging from 4,500 to 6,500 m²/ha and LRV values between 870 and 1,800 m³/ha, representing approximately 39% of the analyzed area. The dark green and light green colors correspond to LWA values ranging from 7,000 to 10,400 m²/ha and LRV values between 2,000 and 5,000 m³/ha, representing approximately 61% of the analyzed area.

Figure 10. SB NDVI vigor map

Table 13. SB NDVI & LWA-LRV correlation

	NDVI map		
Colour	Value	Surface (ha)	Percent
Green	0.19-0.51	3.58	60.37%
Orange	0.06-0.16	2.35	39.62%
		5.93	
	Leaf Wall Area		
	Number of vines	Interval (m²/ha)	Percent
	48	6956-10434	62.33%
	29	4347-6521	37.66%
	77		
	Leaf Row Volume		
	Number of vines	Interval (m³/ha)	Percent
	46	1956-5021	59.74%
	31	869-1847	40.26%
	77		

As a final point of the study, a SWOT analysis has been compiled for the proposed theme.
Strengths: Maps obtained through passive remote sensing technology represents a source of information for the vineyard area and the evolution of a plantation.
Easy and rapid evaluation of cultivated areas without knowing the vineyard's history. A useful tool for choosing an optimal harvest date. Better understanding of plot variability, allowing for more precise implementation of necessary uniformization measures. Image resolution obtained surpasses satellite quality and is much more efficient than human eye observation. Weaknesses: Applicability may be challenging to understand and implement regarding the current processes in Romanian viticulture. An emerging technology in its early stages of adoption in agriculture. Very low interest in research regarding the use of new remote sensing technologies. Very low theoretical knowledge base among personnel who could use this technology in a winery.
Opportunities: Wineries that understand and use drone technology and remote sensing will have a significant competitive advantage in the coming years. Once the applicability of drone data collection is understood, working with drones for phytosanitary spraying will be easily implementable. A very new research branch with enormous exploitation potential. Future studies could explore relationships between NDVI and grape pH before harvest, harvested quantity and the weight of 100 berries.
Threats: The need for winery personnel to undergo retraining for use of new technologies. The primary need for wineries to solve already known problems or at least those easier to

identify. Initial significant investments in hardware, software, or remote sensing services may seem unsustainable or unnecessary. With the development of remote sensing infrastructure, wineries will need to stay technologically up-to-date with other precision viticulture equipment.

CONCLUSIONS

The paper explores the integration of passive remote sensing technology in Romanian viticulture, highlighting its strengths in providing valuable information for vineyards and efficient evaluation of cultivated areas. While presenting promising opportunities for wineries adopting this technology, the analysis emphasizes the need to address challenges such as applicability concerns, low research interest, and the necessity for personnel retraining.

Continued research through more complex analyses for a more detailed understanding involve the following proposals. Demonstrating the correlation of the NDRE vegetation index with leaf nitrogen levels and, consequently, photosynthesis performance. Planning pruning during the dormant period based on NDVI map data to establish the load for the following year. Correlating NDVI and NDRE index maps, resulting in a third index, CCCI (Canopy Chlorophyll Content Index), to identify areas with high potential of disease. Efficientizing phytosanitary treatments by administering substances in quantities according to the NDVI map. Applying selection of sampling points before harvest based on vegetation indices. Plotting the harvest for differentiation of grapes into different quality tiers and vinifying them separately. Understanding soil structure and texture through extracting vegetation indices over successive periods of multiple years. Identifying and demonstrating a crop estimation model using passive remote sensing techniques.

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INFLUENCE OF HIGH-POWER ULTRASOUND TREATMENT ON RED WINE QUALITY PARAMETERS

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Abstract

The objective of this study was to assess the impact of applying high-power ultrasonic treatment (HPU) on crushed Merlot grapes, at a laboratory scale, on the phenolic matrix of red wines, particularly anthocyanins, which are crucial for color, stability, and sensory profile. The ultrasonic treatment (US) was carried out using two amplitudes, 70% and 90%, and three treatment times, namely, 3, 4, and 5 minutes, while maceration was conducted via sequential extraction after 3, 5, and 7 days. After a bottling period of three months, there was a decrease in total polyphenol content observed compared to the content found at the end of maceration. Treatment with ultrasound caused significant variations in the optical density at 420, 520, and 620 nm, and in the content of monomeric anthocyanins. All the sonicated samples, including those extracted after three days of maceration, exhibited significantly higher color intensity values than the maximum color intensity value in the untreated samples. It is noteworthy that the change in color was a positive outcome of this treatment. The Random Forest algorithm was used to identify the most distinct variables among wines. The most significant variable was found to be the total polyphenol content, followed by antioxidant capacity and the color intensity of the wines. The algorithm grouped all the samples into 5 clusters based on three fixed factors that influenced their characteristics: amplitude, treatment time, and maceration duration. Based on these results, it can be inferred that the effects of ultrasound treatment vary significantly depending on the parameters used.

Key words*: high-power ultrasound treatment, red wines, bioactive compounds, chromatic characteristics*

INTRODUCTION

One of the world's earliest known alcoholic beverages is wine. Red wine can be considered a major dietary source of polyphenols due to its generally high polyphenol content (Castaldo et al., 2019). Essential substances called polyphenols give wine its color, flavor, and aroma as well as some possible health advantages (Banc et al., 2014). Red wine's phenolic content can be broadly classified into two groups: non-flavonoids, which include phenolic acids like benzoic, caffeic, and cinnamic acids and stilbenoids like resveratrol, and flavonoids, which include anthocyanins and tannins that contribute to the color and mouthfeel of the wine, flavan-3-ols (or catechins), flavonols, and their derivatives (Gutiérrez-Escobar et al., 2021). Nonetheless, flavonoids account for the majority of wine's phenolic component. Of these, the grape's stems, seeds, and skins account for up to 90% of the phenolic content found in red wine (Nemzer et al., 2021). One-year-old red wine's polyphenol composition is composed of approximately 60–80% polymeric polyphenols, 10-15% anthocyanidins, 5–10% dimer procyanidins, 5-8% catechins, 3-6% phenolic acids, less than 1% flavonols, and less than 0.3% resveratrol, among other particular components (Buljeta et al., 2023). Red wine's phenolic components are known to provide a number of health advantages, including anti-inflammatory, cardioprotective, anticarcino-genic, neuroprotective, and gut microbiota impacts (Nardini, 2022). Many variables, including grape variety and age, pre-fermentation techniques, fermentation and aging circumstances, and technological procedures, influence the kind of polyphenols present in red wine (Luzzini et al., 2021). It is also crucial to remember that these elements may interact in intricate ways to affect how a particular wine's ultimate polyphenol profile is determined. Accordingly, the precise function and influence of polyphenols can change based on things like the kind of polyphenols present, how long they macerate for, and the desired qualities of the finished product (Gómez-Plaza et al., 2020). Wines with good chromatic quality must have a good dispersion of the phenolic compounds from the grape to the must or must-wine. This extraction process, which is based on the disintegration of the grape skin and seed cell walls, is carried out during the maceration step to attain the release of the intended compounds into the medium. As such, the quality of the wine is influenced by the length of the maceration process (Alencar et al., 2018). Ultrasound-assisted extraction (Aadil et al., 2015; Yusoff et al., 2022), microwave-assisted extraction (Khan et al., 2022), cold plasma (Ahmadian et al., 2023; Heydari et al., 2023), supercritical fluid extraction (Molino et al., 2020), pressurized liquid extraction (Zia et al., 2022), high-voltage electric discharge (Molino et al., 2020), pulse electric field extraction (Ranjha, Kanwal, et al., 2021), microfluidization (Mukhtar et al., 2022), and enzyme-assisted extraction (Noranizan et al., 2020) are some of the advanced techniques for extracting plant bioactive compounds from foods and food-related matrices. These improved procedures are 32-36% more efficient, using roughly 15 times less energy and yielding higher-quality extracts (Sridhar et al., 2021). A technique known as high-power ultrasound (HPU) makes use of sound waves with frequency higher than 20 kilohertz (Ali et al., 2023; Hussain et al., 2023; Ranjha, Irfan, et al., 2021). The fundamental way that ultrasound affects a fluid is by increasing the hydrostatic pressure that already exists in the medium with acoustic pressure (Patist & Bates, 2011). The HPU's extraction capacity and the subsequent physico-chemical quality of the wines have been the subject of highly encouraging results in previous studies on red grapes and wines of various types (Q. A. Zhang et al., 2023).

Research has shown that ultrasound can be a valuable tool in winemaking, particularly in the extraction of polyphenols from grapes (Gómez-Plaza et al., 2020; Plaza et al., 2019). Studies have found that ultrasound treatment of grape marc can enhance the accumulation of polyphenols and anthocyanins, as well as influence the quantity of monomeric fraction of anthocyanins (Khmelev et al., 2015). High-power ultrasounds have been shown to modify the physical characteristics of grape skin, facilitating phenolic extraction and improving wine chromatic characteristics (Pérez-Porras et al., 2021). The application of high-power ultrasounds during red wine vinification has been found to be effective in increasing the extraction of polyphenols, particularly in certain grape cultivars (Bautista-Ortin et al., 2017). These findings suggest that ultrasound can play a significant role in enhancing the quality and characteristics of wine. The International Organization of Vine and Wine decided after much investigation that this technology might be used in wineries in 2019 (OIV - International organisation of vine and wine, 2021).

However, the following concerns regarding basic research and technological implementtation in industry need to be addressed. The different research groups used very different ultrasonic equipment, studied very different parameters, and obtained very different results, so they cannot be used as a standard for future research.

The objective of this study was to assess the impact of applying ultrasonic treatment (US) on crushed Merlot grapes, at a laboratory scale, on the phenolic matrix of red wines, particularly anthocyanins, which are crucial for color, stability, and sensory profile. Furthermore, to identify the most distinguishing variables among wines, we utilized the Random Forest clustering algorithm that indicates the most significant variables for grouping the samples in descending order.

MATERIALS AND METHODS

Grapes

The experimental study used about 100 kg of Merlot-varietal red grapes from the 2019 harvest. At the Pietroasa-Istrita Viticulture and Winemaking Research and Development Station in Buzau, Romania, the grapes were gathered by hand. The grapes were harvested at 29.8 °Brix, which is considered technological maturity, and were then promptly transferred to the lab for processing. For the investigation, only sound grape bunches selected at random were used.

Ultrasonic instrument

All experimental procedures were sonicated using SONIX VCX750, a probe-style ultrasonic apparatus from Sonics and Materials Inc. in Newtown, USA (SONIX VCX750, Sonics and Materials Inc., Newtown, USA). One way to express the amplitude is as a percentage, with values between 10% and 100%. The device operates at a frequency of 20 kHz and has an ultrasonic power of 750 W. The 20-100 kHz range is the most often utilized frequency range for extraction techniques.

Preparation of the sample

Samples of 1000 grams of 2019 vintage Merlot red grapes were prepared for laboratory testing. Sixty specimens, each weighing 100 grams, were randomly selected, de-stemmed, manually ground, and subjected to ultrasonic probe treatment. After sonication, we created six 1000 g samples, each containing ten 100 g specimens. The characteristics of each sample are represented by the average of the conclusions drawn from its ten instances. Ten specimens weighing 100 g each were used as the untreated control sample (C) since they had not undergone ultrasonic treatment. The specimens were evaluated independently. To treat the specimens, a 100 mL Pyrex glass beaker with a counter current water-cooling jacket was used. The cooling water was maintained at a consistent temperature of 19 °C. The acoustic amplifier was positioned 20 mm above the bottom of the container to ensure consistent treatment. The untreated sample (Control, C) and the ultrasound-treated samples underwent microvinification. After maceration, samples were sequentially extracted at 3 (D3), 5 (D5), and 7 (D7) days, pressed, and then underwent alcoholic fermentation. To ensure winemaking control, 20 g of Viniferm *Saccharomyces cerevisiae* Agrovin, Spain, fermentation yeast was added per 100 liters. The temperature was monitored daily during alcoholic fermentation, with variations ranging from 19.7 to 21.3°C. After completing alcoholic and malolactic fermentation, the samples were separated from the yeasts and clarified using 30 g of bentonite per 100 liters. Following clarification, the samples were cold-stabilized at 9°C before being bottled. All experiments were conducted in this manner.

Total polyphenolic content and anthocyanins determination

The Folin-Ciocalteu spectrophotometric method was used to calculate the total polyphenolic content (TPC), which was then expressed as micrograms of gallic acid equivalents per milliliter (μg GAE/mL) using gallic acid as a reference (Singleton et al., 1999). A 0.5 mL sample was treated with 1.25 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), which had been diluted 1:10 (v/v) with distilled water. After the mixture had been incubated for five minutes at room temperature, one milliliter of Na_2CO_3 60 g/L was added. After 30 minutes of incubation at 50 °C, the sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany). The calibration curve was made using gallic acid as the standard, which was utilized in values ranging from 5 to 250 g GAE/mL.

Monomeric anthocyanins (MA) were found using the pH differential method (Lee et al., 2005). To make two dilutions of the same sample, 1 mL of wine was combined with 14 mL of potassium chloride buffer (0.025 M, pH 1.0) and 14 mL of sodium acetate buffer (0.4 M, pH 4.5). The absorbance at 520 and 700 nm was measured against deionized water after 15 minutes at room temperature. The results were expressed in milligrams (mg CGE/L) of cyanidin-3-glucoside equivalents per liter. The total anthocyanin content of the samples was calculated using a molecular weight of 449.2 g/mol and a molar absorbance coefficient of 26,900 L/mol x cm.

Ferric reducing antioxidant power (FRAP) assay

The FRAP test methodology was built upon the Benzie and Strain approach (Benzie & Strain, 1996). Two stock solutions were used: 20 mM $FeCl_3$ x $6H_2O$ solution in 300 mM acetate buffer (pH = 3.6) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl. The working solution was prepared by mixing acetate buffer, TPTZ solution, and 10 mL of $FeCl_3$ x $6H_2O$ solution. It was then heated to 37 °C before to use. Prior to analysis, a 0.5 mL aliquot of the diluted wine samples was allowed to react with 2.5 mL of the working solution for 30 minutes at 37 °C. The wine samples had been diluted 1:50 (v/v) with distilled water.

Chromatic characteristics

The intensity of color (IC) and hue (N) of the samples were determined using a spectrophotometric method. The intensity of color was calculated by summing the absorptions at wavelengths 420, 520, and 620 nm with a 1 cm optical path. The hue was expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm (OIV (International organisation of vine and wine), 2021).

Statistical analysis

The results, which were all measured in triplicate, are presented as mean ± standard deviation (SD). Statistical analysis was performed using DESIGN-EXPERT® VERSION 13 software from Stat-Ease Inc. (Stat-Ease Inc., 2020). To assess statistically significant differences between samples, we used one-way ANOVA with post hoc analysis employing the HSD Tukey test. The Pearson correlation coefficient was computed using JASP software version 0.17.1 (JASP Team, 2023) for TPC, MA, FRAP value, IC, and N. The wines were grouped based on the parameters of ultrasound treatment using Random Forest Clustering (JASP Team, 2023). This algorithm partitions data into distinct clusters, with each observation belonging to only one group.

RESULTS AND DISCUSSIONS

Following destemming and crushing, the samples treated with ultrasound and the control sample underwent microvinification. The sequential extraction method was used to carry out the maceration operation over a period of 3, 5, and 7 days.

After fermentation and bottling, the purpose was to assess the impact of high-power ultrasound treatment parameters: amplitude (A) and treatment time (t) on wine quality parameters, specifically bioactive compounds such as TPC (total polyphenol content), MA (monomeric anthocyanin content), FRAP value, color intensity (IC), and hue (N) in the sonicated samples in comparison to the untreated sample (C). The study analyzed the above parameters on untreated samples (C) after 3 (C3), 5 (C5), and 7 (C7) days of maceration. Additionally, the study examined samples that underwent ultrasound treatment at 70% and 90% amplitude for 3, 4, and 5 minutes after 3, 5, and 7 days of maceration. The samples were labeled as follows: SW70/3 (A: 70%; t: 3 min), SW70/4 (A: 70%; t: 4 min), SW70/5 (A: 70%; t: 5 min), SW90/3 (A: 90%; t: 3 min), SW90/4 (A: 90%; t: 4 min), and SW90/5 (A: 90%, t: 5 min). Each sample was replicated three times.

Analytical determinations performed on the samples treated with ultrasound and control samples (untreated), subjected to different durations of maceration of 3, 5, and 7 days, respectively, and microvinified and bottled for three months showed interesting results (Table 1). As these are red wines, it is important to consider the potential impact of ultrasonic treatment on the coloring matter.

The sonicated samples showed distinct chromatic features and higher concentrations of bioactive chemicals from the beginning of the maceration than the untreated samples. This difference persisted until the end of the maceration (Maier et al., 2023; Margean et al., 2020).

Effects of ultrasound treatment on wine parameters

After a bottling period of three months, both the wines made from crushed grapes subjected to ultrasonic treatment and the control wines showed a decrease in total polyphenol content compared to the content found at the end of maceration. However, the TPC values found in the sonicated samples remained higher than those of the controls, regardless of the duration of maceration (Table 1).

The total polyphenol content registered a maximum for all samples at the end of maceration and a decrease after bottling for all samples, with values ranging from 1.12% for sample SW70/3 extracted after 5 days (1169.23 µg GAE/mL at the end of maceration) to 64.62% for sample C3 (870.32 µg GAE/mL at the end of maceration).

High-power ultrasound applied to grapes or during winemaking has been shown in previous research to produce a greater extraction yield of

TPC (Ferraretto et al., 2011; Pérez-Porras et al., 2021). Other authors have reported no significant degradation of polyphenols after sonication of red wine (Natolino & Celotti, 2022).

Table 1. Effects of the ultrasonic treatment on the parameters of the wine

Sample	Maceration (days)	TPC (µg GAE/mL)	MA (mg CGE/L)	FRAP (µM Fe^{2+}/mL)	IC	N
C3	3	307.91±2.36	63.19±1.6	39.1±2.4	4.8±0.3	0.56±0.02
SW70/3		893.47±19.6	129.51±3.6	55.97±0.9	16.86±0.88	0.6±0.03
SW70/4		876.24±12.1	153.15±3.9	57.70±2.25	16.87±1.1	0.6±0.01
SW70/5		1 115.26±15.4	131.07±2.8	55.22±1.7	16.65±2.3	0.6±0.01
SW90/3		946.24±15.2	137.59±6.5	61.57±2.3	15.93±1.1	0.57±0.02
SW90/4		1 648.75±24.9	152.04±4.6	69.00±2.3	17.56±0.83	0.61±0.08
SW90/5		1 333.94±16.98	158.63±2.2	68.13±2.04	15.98±1.47	0.54±0.01
C5	5	415.76±3.8	95.99±3.7	54.64±2.6	5.63±0.76	0.57±0.02
SW70/3		1 156.14±11.9	138.79±2.6	52.77±1.6	11.6±1.2	0.57±0.05
SW70/4		1 094.92±13.1	171.06±2.5	52.07±2.1	8.47±1.5	0.72±0.09
SW70/5		1 304.79±12.4	125.51±6.3	57.46±2.8	14.36±1.4	0.64±0.02
SW90/3		1 159.05±11.3	123.68±5.6	57.61±3.0	11.36±1.2	0.67±0.04
SW90/4		1 243.58±10.8	126.74±4.9	68.52±4.9	11.49±1.3	0.67±0.05
SW90/5		1 605.02±21.7	122.86±4.8	56.06±2.7	15.13±0.9	0.63±0.05
C7	7	439.08±8.9	119.18±6.1	46.65±3.7	6.44±1.1	0.58±0.1
SW70/3		1 043.58±18.4	121.63±3.8	58.89±2.1	13.66±1.1	0.64±0.07
SW70/4		1 269.82±13.8	156.28±4.2	61.39±1.8	9.06±0.8	0.65±0.03
SW70/5		1 418.47±21.3	121.66±3.6	68.04±2.9	14.76±1.2	0.65±0.07
SW90/3		1 261.07±19.2	159.61±4.1	68.84±1.9	13.53±1.0	0.68±0.02
SW90/4		1 296.05±23.1	200.36±2.7	59.91±1.8	11.92±1.2	0.70±0.04
SW90/5		1 596.28±25.4	132.43±2.8	61.36±1.9	16.54±0.7	0.67±0.01

Untreated sample (C); SW70/3 (A: 70%; t: 3 min), SW70/4 (A: 70%; t: 4 min), SW70/5 (A: 70%; t: 5 min), SW90/3 (A: 90%; t: 3 min), SW90/4 (A: 90%; t: 4 min), and SW90/5 (A: 90%, t: 5 min); Total polyphenolic content (TPC); Monomeric anthocyanins content (MA); Ferric reducing antioxidant power (FRAP); Intensity of color (IC); Hue (N); The results are expressed as the mean value of the three replicates ± the standard deviation (SD).

Moreover, high-power ultrasound has been shown to have a significant impact on the phenolic structure of red wines, particularly in accelerating the aging process (Ferraretto & Celotti, 2016). It can also enhance the extraction of polyphenols from grapes during winemaking, with varying effects depending on the grape variety (Gambacorta et al., 2017). However, the specific effects on polyphenols in wine during storage are still being explored (Q. Zhang & Wang, 2017b).

As can be observed in Table 1, the treatment with HPU caused significant variations in the optical density (420, 520, and 620 nm) and in the monomeric anthocyanin content. After a period of three months of bottling, there was a significant decrease in the color intensity values of the wines for all the samples, compared to the values obtained at the end of the maceration, with the greatest decrease being recorded for the sample SW90/5 extracted after 5 days (24.85 at the end of maceration).

However, based on the data analyzed, it is evident that the ultrasonic treatment had a positive effect on the color content. All ultrasound samples, including those extracted after 3 days of maceration, recorded significantly higher color intensity values compared to the untreated samples (C7), ranging from 31.52% (SW70/4 sample, extracted after 5 days) to 172.67% (SW70/4 sample, extracted after 3 days). Other authors found that ultrasound treatment affects the evolution of color properties and major phenolic compounds in wine during storage, showing similar effects in treated and untreated wines, with quicker changes observed in treated wines (Q. Zhang & Wang, 2017a). According to additional research, it was found that the control samples that were macerated for 48 hours had the lowest color intensity. However, the differences from the other control wines were not statistically significant. Furthermore, the color intensity of the sonicated samples macerated for 48 hours was not significantly different from the control wine that was exposed to the skin for 7 days (Pérez-Porras et al., 2021).

The monomeric anthocyanin content was also measured for all samples immediately after maceration stage. After three months of bottling, the quantities present in the wine samples were smaller, with a decrease between 27.35% and 73.03%. The smallest decrease was observed in the case of sample SW90/4, which was extracted after 7 days (275.78 mg CGE/L at the end of maceration). This decrease in monomeric anthocyanin content is likely caused by factors such as polymerization with other phenolic compounds and oxidation reactions.

The influence of ultrasonic treatment on the monomeric anthocyanin content of wine samples is similar to its effect on their color content. The untreated samples had the highest content of monomeric anthocyanins in the C7 sample. However, the sonicated samples showed significant variations in the monomeric anthocyanin content compared to the untreated samples (Table 1). The increase ranged from 2.05% (sample SW70/3, extracted after 7 days) to 68.1% (sample SW90/4 min, extracted after 7 days). According to (Dalagnol et al., 2017), ultrasound-assisted extraction was found to accelerate the rate of anthocyanin extraction. It is worth noting that free anthocyanins are the primary source of red color in young red wines, despite the instability of monomeric anthocyanins. To stabilize these anthocyanins, one of the main methods is to condense them with tannins to generate stable anthocyanin/tannin adducts. It has been estimated that approximately 25% of the anthocyanins may undergo polymerization with flavonoid molecules towards the end of the alcoholic fermentation process. Following a year, this percentage is observed to increase to over 40% (He et al., 2012a, 2012b).

The antioxidant capacity increased for all samples after the bottling period compared to the values found at the end of maceration. The increase in antioxidant activity ranged from 3.52% for sample SW90/5, which was extracted after 7 days (59.27 µmol Fe^{2+}/mL at the end of maceration), to 47.1% for sample SW70/4 min, which was extracted after 5 days (52.48 µmol Fe^{2+}/mL at the end of maceration). Nevertheless, it appears that the sonicated samples may have contained a notable quantity of phenolic compounds as a result of the US treatment, which led to elevated FRAP values.

Previous studies have indicated that the phenolic composition of wine can be influenced by the winemaking process. Moreover, a significant correlation has been found between the phenolic composition and the ferric reducing antioxidant power of samples (Lingua et al., 2016). It has also been concluded that the antioxidant capacity of wines is more closely related to the individual phenolic compounds present in the wine, rather than the total phenolic content (Banc et al., 2020).

The shade of wines after three months of bottling varies between 0.54 for sample SW90/5, extracted after 3 days, and 0.72 for sample SW70/4, extracted after 5 days (Table 1). A smaller shade indicates a larger red component compared to yellow, while high shades indicate an orange color. It is likely that anthocyanins degrade and condense with tannins, forming complexes that contribute to color stabilization.

Correlation of maceration duration, amplitude level, and sonication time

The statistical analysis of the data reveals that the investigated parameters were affected by three factors: amplitude level, ultrasonic treatment time, and maceration duration, either independently or in combination. The analysis of variance conducted on the analytical parameters for different maceration durations, amplitude, and treatment time conditions showed significant differences in the total content of polyphenols, monomeric anthocyanins, antioxidant capacity, color intensity, and hue of wines. The evolution of the analyzed parameters appears to be influenced by changes in maceration duration, percentage of amplitude, and treatment time. It is worth noting that the correlation coefficient (R) is 0.791 for CTP and 0.208 for AM. Additionally, the antioxidant activity also appears to vary, with a correlation coefficient of 0.518. The adjusted R^2 used for the three predictors: maceration duration, amplitude and treatment time shows that they can predict: 60.4% of the variation in results obtained for

CTP; 46.3% of the variation in results obtained for AM; 22.4% of the variation in FRAP results; 26.2% of the variation in results obtained for IC; and 22.3% of the variation in results obtained for hue. Previous research has also established a direct proportionality between the amplitude of the transducer and the intensity of the ultrasound. The sonochemical effects of the ultrasound were observed to increase with increasing intensity and amplitude (Mason & Lorimer, 2002). ANOVA shows that the model is significant, and the predictors introduced into the model, both alone and in the case of interaction between them, significantly influence ($p<0.05$) the total content of polyphenols, monomer anthocyanins, and FRAPs (Table 2).

Table 2. Exploring the impact of various factors on wine parameters

	p-value				
Factors	**TPC**	**MA**	**FRAP**	**IC**	**N**
Amplitude (%)	<0.001	<0.001	<0.001	<0.05	0.564
US treatment time (min)	<0.001	<0.001	<0.05	<0.001	<0.05
Maceration time (days)	<0.001	<0.001	<0.001	<0.001	<0.001
Maceration time *Amplitude	<0.001	<0.001	<0.001	<0.05	0.162
Maceration time *US treatment time	<0.001	<0.001	<0.001	<0.001	0.549
Amplitude *US treatment time	<0.001	<0.001	<0.001	<0.05	0.288
Maceration time *Amplitude *US treatment time	<0.001	<0.001	<0.001	0.976	0.164

Significance of each parameter (p-value); $p<0.05$ statistically significant and $p<0.001$ statistically highly significant; Total polyphenolic content (TPC); Monomeric anthocyanins content (MA); Ferric reducing antioxidant power (FRAP); Intensity of color (IC); Hue (N).

The color intensity appears to be minimally impacted by the interaction between amplitude level, treatment time, and maceration duration. However, it is worth noting that the hue of wines is significantly influenced by treatment time and duration of maceration.

Identifying the most distinguishing variables between wines by grouping them

Random Forest clustering is a hard-partitioning algorithm that divides data into multiple clusters (groups), with each observation belonging to only one group (JASP Team, 2023). This clustering approach uses the Random Forest algorithm in an unsupervised manner, with the output variable 'y' set to NULL.

The wine dataset contains the results of analytical determinations of wines produced according to the traditional technology (control samples) and wines obtained from crushed grapes treated with ultrasound before the maceration stage. Two types of wine are represented in the 63 samples (21 samples analyzed in triplicate), with the results of 5 analytical determinations recorded for each sample. The variables recorded are TPC (µg GAE/mL), MA(mg CGE/L), FRAP (µM Fe^{2+}/mL), IC and N.

The purpose of using the Random Forest algorithm is to identify the most distinct variables among wines. Specifically, the Random Forest cluster model is optimized against its BIC value, which can be inspected in the Elbow Curve Diagram.

Table 3 and Table 4 present summary and performance statistical data, such as the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) of the model.

Table 3. Statistical data on cluster grouping

Number of clusters	Number of samples	R^2	AIC	BIC	Silhouette
5	63	0.638	162330	215910	0.220

Determination coefficient (R^2); Akaike Information Criterion (AIC); Bayesian Information Criterion (BIC); The model is optimized with respect to the BIC value.

AIC is an estimator of prediction error and provides a means for model selection. BIC is a criterion for model selection from a finite set of models. The preferred model is the one with the lowest BIC. BIC is closely related to the AIC criterion and is partly based on the probability function. When the models match, adding parameters can increase the probability, but this can lead to overlap. Both BIC and AIC introduce a penalty term for the number of parameters in the model to solve this problem. However, the penalty term is higher in BIC than in AIC. The silhouette value of the model ranges from -1 to 1, with 1 representing a perfect score. The sum of squares of each cluster indicates the spread within the cluster, while R^2 indicates the amount of variance explained by the model (Table 4). Silhouette scores describe the degree of separation between groups.

Table 4. Grouping and sizes of clusters

Cluster	1	2	3	4	5
Dimension (number of samples)	9	11	16	14	13
Sum of squares within the group	19170	5998	36059	28188	22917
Silhouette score	0.468	0.498	0.003	0.108	0.177

Table 5 displays the Random Forest algorithm's ranking of variables in descending order of importance for grouping samples. The most significant variable is the total polyphenol content, followed by antioxidant capacity and the color intensity of the wines.

Table 5. Importance of variables in sample grouping

Parameter	Importance value
Total polyphenol content	13796
Antioxidant capacity	12996
Color intensity	12952
Monomeric anthocyanins	12628
Hue	10089

The Elbow diagram indicates the point at which adding another cluster would be unnecessary, also known as the kink in the curve. The red dot represents the minimum BIC value, which is the metric optimized by the model (Figure 2).

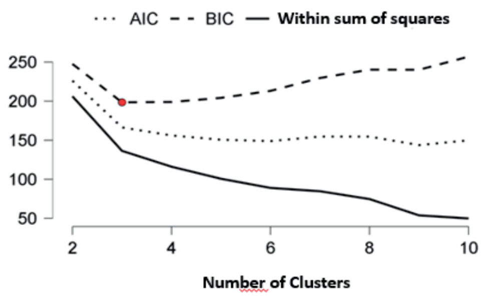

Figure 2. Elbow Diagram

Figure 3 displays the two-dimensional t-SNE graph, which provides a comprehensive overview of the various characteristics determined for the samples. However, the disadvantage is that the axes become uninterpretable. This t-SNE graph illustrates how the different clusters are grouped together. Moreover, Figure 3 displays the sample grouping into clusters based on three fixed factors that influenced their characteristics: amplitude, treatment time, and maceration duration.

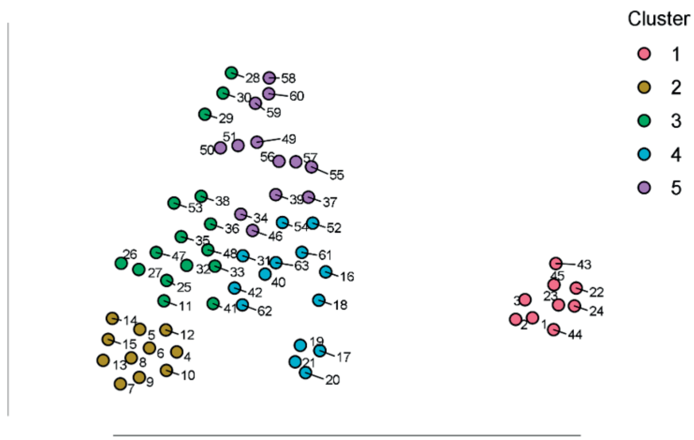

Figure 3. Two-dimensional t-SNE plot illustrating clustering of samples

Compared to all samples that underwent ultrasonic treatment, the control samples, regardless of maceration duration, recorded significantly lower values for all determined

parameters (refer to Table 1). These control samples were grouped in a separate cluster (Cluster 1).
A cluster (Cluster 2) was identified among the samples treated with ultrasound. This cluster was influenced by both the lower amplitude (70%) used during treatment, regardless of the treatment time (3, 4, or 5 minutes), and the increased amplitude (90%) used during a short treatment time (3 minutes). As a result, significantly lower values were recorded for all determined parameters compared to the other samples subjected to ultrasonic treatment. Cluster 3 confirms the positive influence of amplitude and treatment time on the properties of wines. It also shows the influence of maceration duration. The samples in Cluster 3 were extracted after 5 days, which is 2 days longer than the samples in Cluster 2. The same cluster also includes another sample that underwent maceration for 7 days but was treated with lower amplitude (70%) for only 3 minutes.
Cluster 4 and Cluster 5 group samples that underwent ultrasonic treatment with increased amplitude (90%) for 4 or 5 minutes, regardless of the duration of maceration, and additionally two samples that were treated with lower amplitude (70%) for 4 or 5 minutes, but with a maceration duration of 7 days. Clusters 4 and 5 recorded the highest values for all analyzed parameters of the wine samples obtained.
It can be inferred from these results that the effects of ultrasound treatment are subject to significant variation based on the parameters employed.

CONCLUSIONS

After a bottling period of three months, a decrease in the total polyphenol content was observed compared to the content found at the end of maceration, both in the wines made from crushed grapes treated with ultrasound and in the control samples, with the values found in the ultrasound-treated samples remaining higher than those of the control samples, regardless of the duration of maceration-fermentation of the latter. However, within each series (D3, D5 and D7), the decrease was influenced by the variation of the ultrasonic treatment parameters. The positive effect of the ultrasonic treatment on the color content can be observed since all the ultrasonically treated samples, including those extracted after 3 days of maceration, showed significantly higher color intensity values than the maximum color intensity value of the control samples (C7), ranging from 31.52% (sample SW70/4, extracted after 5 days) to 172.67% (sample SW70/, extracted after 3 days). In conclusion, the ultrasonic treatment significantly modified the color of the wine, an effect that is very beneficial to the quality, especially for young wines, where color is one of the most important evaluation factors. The monomeric anthocyanin content of the sonicated samples showed significant variation compared to the maximum of the untreated samples, with an increase ranging from 2.05% (sample SW70/3 min, extracted after 7 days) to 68.1% (sample SW90/4 min, extracted after 7 days). The maximum anthocyanin content of the untreated samples was determined for the one extracted after 7 days. The data, subjected to statistical analysis, show that amplitude level, ultrasound treatment time and maceration duration significantly ($p<0.05$) influence the total polyphenol content, monomeric anthocyanins and antioxidant capacity. In addition, colour intensity is not significantly influenced by the interaction between amplitude level, treatment time and maceration time, while wine hue is significantly influenced only by treatment time and maceration time. Furthermore, to identify the most distinguishing variables among wines, we utilized the Random Forest clustering algorithm. The Random Forest algorithm indicates the most significant variables for grouping the samples in descending order. Our results display that the total polyphenol content is a distinguishing feature, followed by antioxidant activity and the color intensity of the wines. All untreated samples, regardless of maceration time, consistently recorded significantly lower values for all determined parameters, in contrast to all samples exposed to ultrasound treatment, and were grouped in a separate cluster. The ultrasound-treated samples were categorized into four clusters, confirming that the effects of ultrasound treatment vary considerably depending on the parameters used (amplitude and time).

However, further research is needed to fully understand the implications of ultrasound on wine color and organoleptic characteristics, as well as the economic feasibility and scalability of implementing high-power ultrasound in winemaking processes.

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TRAINING TYPE, CROP LOAD AND SHADING EFFECT ON QUALITY COMPONENTS OF ITALIAN RIESLING GRAPE VARIETY

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Abstract

The study investigated the influence of crop load and grape berry exposure on the characteristics of Italian Riesling grapes from the Recaş vineyard, Timiş County, Romania. The research focused on vines managed under the Guyot training system, utilizing both single and double cordons, arranged in north-south rows. Evaluation of grape exposure encompassed strategies such as: complete (100%) and partial (50%) leaf thinning around the bunches. Sixteen distinct plots were established, organized into four blocks, to examine various management practices and thinning techniques. Vine vigor was assessed through measurements of pruning and leaf area, with harvesting schedules adjusted to achieve similar °Brix values across plots. Following berry sampling and processing, grape juice analysis was conducted, revealing that vines trained double Guyot generally exhibited superior grape yield. However, higher crop loads were associated with reduced leaf area, resulting in delayed veraison and impacting sugar accumulation. Crop load had a significant influence on grape berry juice acidity and pH level, depending also of training system. Overall, the findings underscored the importance of the leaf surface-to-grape yield ratio as a critical determinant of grape yield and juice quality.

Key words: *Italian Riesling, Guyot training, leaf area, crop load, °Brix.*

INTRODUCTION

Training systems have an impact on canopy development, airflow, sunshine exposure, and vineyard management and microclimate, all of which adversely impact berry development and grapevine physiology (Kraus et al., 2018). The Guyot is one of the most common vine training systems in viticulture, noted for its simplicity and flexibility to different terroirs and grapevine varieties (Lanari et al., 2022). Guyot pruning develops an open canopy, which improves airflow and sunlight within the canopy, boosting photosynthesis and grape maturation (Del Zozzo & Poni, 2024).). According to studies, vines with Guyot training accumulate more sugar, resulting in higher Brix level and higher potential alcohol concentration in the wines (Sabbatini and Howell, 2010). Furthermore, better solar exposure result in better phenolic maturity, wine mouthfeel and colour (Minaar et al., 2020).

Crop load management is a key component of viticulture, given that it affects grape berry composition as well as final wine quality (Reynolds, 2022). Pruning, thinning, and cluster removal can all be used to control the number of grape clusters or berries on a vine (Reeve et al., 2018). Understanding the impact of crop load on grape berry composition is critical for vineyard management and producing optimal wine qualities (Luna et al., 2017). Crop load has a considerable impact on sugar accumulation in grape berries. Large crop load frequently results in less sugar accumulation resulting from increased competition among vine organs for nutrients and water (Tangolar et al., 2019). In contrast, lower crop load may stimulate higher amounts of sugar in berries, resulting in higher potential alcohol concentrations in wine (Nistor et al., 2021). According to research studies, higher crop load result in grapes with lower acidity levels (Kliewer & Dokoozlian, 2005). This is due to dilution effects, in which limited resources (water and nutrients) are dispersed among a larger number of berries, resulting in lower acid levels (Brunetto et al., 2020). Crop load adjustment can affect the concentration of phenolic compounds, including anthocyanins and tannins in grape berries. Research has shown that modest crop loads can produce appropriate phenolic maturity, which results in balanced wine phenol compounds (Mori et al. 2007). However, overly high or low crop load can cause phenolic composition imbalances, which impact wine quality (Poni et al., 2018). Moderate crop loads can increase the development of favourable aroma components, hence increasing wine flavourful complexity (Cataldo et al., 2021). In contrast, high crop loads may result in diminished fragrance richness and complexity, whilst low crop loads may result in too powerful flavours (Previtali et al., 2021). Shading can have a variety of impacts on grape physiology and metabolic activity, thereby influencing wine quality. Understanding these effects is essential for vineyard management strategies with the goal to improve grape quality and wine qualities (Basile et al. 2015). Shaded berries have lower levels of sugar than those exposed to direct sunlight. This is due to lower photosynthetic activity in shaded places, which reduces carbohydrate synthesis and the accumulation of sugar in the grape berries (Garrido et al., 2018). Shading also influences grape berry acidity, which is important for wine flavour and balance. Shaded berries have lower acidity levels than sun-exposed berries, due to lower malic acid decomposition in shaded places, which lead to higher malic acid levels and lower tartaric acid concentrations (Michelini et al., 2021). Anthocyanins, flavonoids, and tannins are essential phenolic chemicals that contribute to wine colour, mouthfeel and flavour (Blancquaert et al., 2019). Canopy shading may alter the synthesis and concentration of these chemicals in grape berries (Ma et al., 2021) According to previous research, shaded berries typically have lesser quantities of phenolic compounds, resulting in lighter-coloured wines with less tannin content and less complexities (Anić et al., 2021).

The aim of the research was to investigate the effects of crop load and grape berry exposure on Italian Riesling grapes and wine from the Recaș vineyard in Timiș County, Romania. Specifically, it focused on vines managed under Guyot training, assessing grape exposure through various leaf thinning techniques and their impact on grape and wine composition.

MATERIALS AND METHODS

Research site

The research carried on during 2020-2022 growing seasons, was located in a vineyard from the western part of Romania, near Timișoara, Romania, namely Recaș vineyards (45.5272° N latitude and 21.1875° E longitude) with its specific terroir and uniformity of the soil type, vine size and age, or training system. The soil type in the Recaș, is mostly loamy and consists of sand, silt, and clay in nearly equal amounts. This soil is noted for its fertility and ability to hold rainwater while providing adequate drainage. Furthermore, the soil is rich in organic matter, which increases its fertility and is widely available and suitable for viticulture, making it a good basis for grape development. The experimental vineyard comprised sixteen plots (organized into four blocks, with each block containing four plots). Each plot represented a unique combination of training method (single or double cordons) and leaf thinning technique (complete or 50%). This configuration allowed for the comparison of various management and thinning techniques. The Italian Riesling vines were trained using the Guyot system (single and double cordons). Single cordons consisted of one main horizontal shoot, while double cordons had two horizontal shoots trained along the trellis wires. The vines were organized into north-south rows to optimize sunlight exposure. Leaf thinning was conducted to regulate sunlight exposure to the grape clusters and promote ripening. Grape exposure was assessed through two different leaf thinning techniques: a. complete (100%) leaf thinning (all leaves surrounding the grape bunches were removed); b. 50% leaf thinning (approximately half of the leaves surrounding the grape bunch were removed). Throughout the growing season, regular monitoring and data collection were conducted. Parameters such as vine growth, fruit development, cluster morphology, and grape ripening were assessed. Sampling was conducted at different stages of grape development, including veraison and harvest. Measurements included: cluster weight, berry weight, total soluble solids (°Brix), acidity levels and pH.

Weather data

Moderate temperatures (average temperature - 12.61°C) throughout the 2020 year and an average of 18.66°C between April to September, have contributed to gradual vine growth and balanced physiological development. The total precipitation amount in the Recaș area for 2020 was 862.3 mm. Adequate water availability has provided essential moisture for vine growth, particularly during critical stages such as bud break, flowering, and fruit set, nutrient uptake and photosynthesis, contributing to the overall vigour and productivity of the grapevine.

The average temperature for the 2021 year in Recaș vineyards was 12.59°C. This moderate average temperature indicates favourable conditions for vine growth and development throughout the year. From April to September, the average temperature rose to 19.23°C. This period corresponds to the growing season for grapevines, during which warmer temperatures support vegetative growth, flowering, and fruit development. The total amount of rainfall in the Recaș area for 2021 was 642.27 mm. While slightly lower than the precipitation recorded in the previous year, this amount still indicates sufficient moisture for vine growth and development. Spring and early summer have seen moderate to adequate rainfall, supporting healthy vine growth. However, there have been periods of dry weather during the late summer and early autumn, which have impacted grape yields and quality.

The weather in the Recaș area during 2022, characterized by moderate temperatures (average 12.74°C during the year and 19.35°C from April to September) and sufficient but slightly lower rainfall (414.8 mm), continued to provide favourable conditions for grapevine development. These conditions contributed to the production of healthy grapevines and high-quality berries, ultimately influencing the characteristics of the wines produced in the region.

Leaf area and grape yield/quality components

Leaf thinning was performed after flowering at berry set beginning, and 3 and respectively 6 basal leaves were removed from each shoot. Six vines were selected at random, both from those with single and double Guyot training. These six vines (totalling twelve in all) were chosen both from the plots with complete leaves thinning in the cluster area and from the plots with only 50% of leaf thinning. From the selected vines, leaves were removed and a sample of about 10% of their fresh weight was measured. A leaf image analysis system (WD-E3 WinDIAS Leaf Image Analysis System - AlphaOmega-Electronics, Maranata-Madrid S.L., Spain) was used to calculate the surface of the leaf/leaves. Grape yield was analyzed by counting and weighing the clusters on each of three selected vines in each plot.

Berry sampling was done starting from the last two weeks of August until berry maturation (around 20°Brix) and harvest (after 15 September for Single Guyot and respectively after 20 of September for double Guyot) in each growing season; every seven days, 50 berries were collected for analysis. In the laboratory, the berries were processed and analyzed for soluble solids (°Brix), titratable acidity and pH. Whole bunches were chosen randomly from the base, middle and top of the shoots on the vine. The collected bunches and berries were placed in labelled plastic bags and sealed and stored in cooler bags for transport.

Grape yield was determined by weighing grapes on a precision scale (Gramme EM-10K, Waagenet, Berlin, Germany). To analyse soluble solids, titratable acidity, and pH, the berries were crushed and blended with a magnetic stirrer (HI310N, Hanna Instruments). After filtration, the juice's soluble solids were assessed using a refractometer (HI96801, Hanna Instruments). Titratable acidity and pH were determined with a pH meter (HI98169, Hanna instruments).

Statistical analysis

Data collected from the sixteen plots were analyzed to evaluate the impact of different training methods and leaf thinning techniques on grape and wine quality. Calculations and statistical analyses were performed using GraphPad Prism software version 8.0 for Windows (GraphPad Software, San Diego, CA, USA). The comparison of means among different groups of numerical variables was conducted using one-way analysis of variance (ANOVA). Due to the non-normal distribution of most data, results are expressed as median, and a p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSIONS

Leaf area and weight

Grapevine training systems are essential in determining vine development, grape yield, and fruit quality (Heuvel et al., 2013). Among the different methods of training, the Single Guyot (SG) and Double Guyot (DG) systems are commonly used, each with their own particular characteristics (Kobayashi et al., 2020).

Table 1. The influence of Single and Double Guyot training on leaf area and weight in Italian Riesling variety

Guyot training	Year	% Thinning	Leaf area/vine (m^2)	Leaf weight/vine (kg)	Leaf area/main shoot (m^2)
Single Guyot	2020	100%	2.12	1.13	0.169
		50%	3.27	1.46	0.251
	2021	100%	1.97	1.06	0.151
		50%	3.11	1.48	0.238
	2022	100%	1.86	0.97	0.143
		50%	2.98	1.31	0.229
Double Guyot	2020	100%	1.46	0.74	0.113
		50%	2.53	1.17	0.194
	2021	100%	1.34	0.67	0.103
		50%	2.47	1.11	0.190
	2022	100%	1.18	0.58	0.097
		50%	2.14	1.03	0.165
p - value (two tailed)			0.0005	0.0005	< 0.0001
SD			0.6921	0.2880	0.0520
SE			0.1998	0.0831	0.0150
CV%			31.42	27.19	30.58%

The small p-value of 0.0005 (Table 1) indicates strong evidence of significant differences in leaf area per vine suggests that factors such as vineyard management practices, soil composition, or environmental conditions likely influence leaf area per vine, as Cataldo et al. (2020) found out in their research. The larger standard deviation of 0.6921 indicates greater variability in leaf area, which means that some vines have substantially higher or lower leaf areas than the average, which can be influenced by factors such as vine health, or local microclimate that affect leaf development, factors also mentioned by Van Leeuwen (2022) in his studies. Moderate to high variability (CV% of 31.42%) was found in the leaf area per vine, which may have implications for vineyard management decisions such as pruning practices for optimize grape yield and quality.

The observed differences in leaf weight per vine are statistically significant (p - 0.0005) that meaning they are likely to have practical implications for vineyard management and grape production. The standard deviation (0.2880) shows that there is moderate variability in leaf weight per vine among vineyard plots, treatments, or conditions under consideration. Understanding the variability in leaf weight per vine is critical in vineyard management since it can reveal information about vine health, vigour, and canopy development (Pereyra et al., 2023). The standard error of 0.0831, suggests that the sample mean leaf weight per vine is likely to be a reliable estimate of the population mean within the study population. The moderate to high variability (CV% of 27.19%) reveals that there are considerable differences in leaf area per main shoot among the samples, implying potential heterogeneity for this variable throughout the investigated samples.

The observed variability ($p < 0.0001$) for leaf area on the main shoot indicates that there are underlying factors or influences (environment, vineyard management, variety genotype) at play that are contributing to the observed differences in leaf area. With a standard deviation of 0.052, it infers that the data points for leaf area from to the main shoot exhibit relatively low variability. With a standard error of 0.015, the sample mean estimations of leaf area relative to the main shoot are very accurate. Results show a coefficient of variation of 30.58% indicates a moderate level of variability in the leaf area from the main shoot. This variability has implications for vineyard management decisions, as it can affect canopy density, sunlight exposure or grape ripening (Gatti et al., 2022).

Table 2. Training system and 100% leaves thinning influence on canopy in Italian Riesling (2020-2022)

Training system	Year	Thinning	Leaf area / vine (m^2)	Leaf weight / vine (kg)	Leaf area / main shoot (m^2)
Single Guyot	2020	100%	2.12^{**}	1.13^{*}	0.169^{*}
	2021	100%	1.97^{*}	1.06^{*}	0.151^{*}
	2022	100%	1.86^{*}	0.97^{ns}	0.143^{*}
Double Guyot	2020	100%	1.46^{*}	0.74^{ns}	0.113^{ns}
	2021	100%	1.34^{*}	0.67^{ns}	0.103^{ns}
	2022	100%	1.18^{ns}	0.58^{ns}	0.097^{ns}

(*for $p < 0.05$, ** for $p < 0.01$, and "ns" for not significant) based on a standard significance level of $\alpha = 0.05$

Across all three growing seasons, SG consistently outperforms DG in terms of leaf area per vine, leaf weight per vine, and leaf area per main shoot (Table 2). The differences observed between the two training systems are statistically significant, suggesting that the choice of training system has a significant impact on leaf characteristics for the Italian Riesling grape variety. Thinning, performed at 100% in both training systems, did not significantly affect the observed differences between SG and DG. While the differences between the two training systems are consistent across years, there is some variability within each system across different years, as indicated by the use of symbols.

Table 3.Training system and 50% leaves thinning influence on canopy in Italian Riesling (2020-2022)

Training system	Year	Thinning	Leaf area / vine (m^2)	Leaf weight / vine (kg)	Leaf area / main shoot (m^2)
Single Guyot	2020	50%	3.27^{**}	1.46^{**}	0.251^{**}
	2021	50%	3.11^{**}	1.48^{**}	0.238^{**}
	2022	50%	2.98^{**}	1.31^{**}	0.229^{**}
Double Guyot	2020	50%	2.53^{*}	1.17^{**}	0.194^{*}
	2021	50%	2.47^{*}	1.11^{*}	0.190^{*}
	2022	50%	2.14^{*}	1.03^{*}	0.165^{*}

(*for $p < 0.05$, ** $p < 0.01$) based on a standard significance level of $\alpha = 0.05$

The statistical analysis reveals consistent trends in leaf area per vine, leaf weight per vine, and leaf area on the main shoot across the three years for both training systems (Table 3). Single Guyot (SG) consistently outperforms Double Guyot (DG) in terms of these measurements, indicating that Single Guyot (SG) training may lead to higher leaf area and weight per vine as well as larger leaf area on the main shoot. The variability within each training system appears relatively stable over the three years, suggesting consistent performance within each system. However, the differences between the two training systems are significant, with SG consistently showing higher values compared to DG.

These findings suggest that the choice of training system can significantly impact leaf area, leaf weight, and leaf area on the main shoot, which in turn may influence grapevine growth, yield, and fruit quality. Winemakers and vineyard managers should consider these factors when selecting the most suitable training system for their vineyards.

Harvest time and berry quality

In order to obtain a similar sugar concentration (around 20 °Brix), the grapes were harvested with a difference of a few days in each growing season, the grapes from SG being harvested earlier.

To analyze the data, were conducted paired t-tests for each variable to compare the means between the Single Guyot (SG) and Double Guyot (DG) training systems at different thinning levels (100% and 50% respectively) for each growing season (2020, 2021, and 2022). The p-values indicate whether the observed differences between the training systems are statistically significant (Table 4). The statistical analysis conducted on the grape yield, bunches per vine, bunch weight, berry weight, total soluble solids (TSS), titratable acidity (TA), and pH levels across different years reveals insightful findings regarding the comparison between the Single Guyot (SG) and Double Guyot (DG) training systems.

Table 4. Comparison of Italian Riesling characteristics between Single Guyot and Double Guyot training systems at different thinning levels across three growing seasons (2020-2022)

Training system	SG	DG		SG	DG		SG	DG		SG	DG		SG	DG		SG	DG	
Year	2020						2021						2022					
Thinning	100%	100%	p	50%	50%	p	100%	100%	p	50%	50%	p	100%	100%	p	50%	50%	p
Grape yield (kg)	2.74	3.13	ns	3.15	3.43	ns	3.05	3.54	ns	3.40	3.87	ns	2.81	3.14	ns	2.98	3.48	ns
Bunches/vine	24.8	32.4	ns	28.3	34.3	**	27.2	33.2	ns	29.7	41.1	ns	26.4	35.6	**	30.1	37.5	**
Bunch weight (g/bunch)	114.2	97.8	**	112.6	100.8	***	112.9	107.3	*	117.4	94.4	**	121.3	89.7	***	119.6	94.1	***
Berry weight (g)	1.52	1.30	ns	1.50	1.35	ns	1.51	1.43	ns	1.57	1.26	ns	1.62	1.20	ns	1.59	1.25	ns
TSS (ºBrix)	20.87	19.87	ns	19.95	20.46	ns	20.06	20.17	ns	20.19	19.43	ns	21.85	21.31	ns	21.23	20.14	ns
TA (H_2SO_4/l)	5.73	6.20	ns	6.23	5.92	ns	6.07	6.18	ns	6.12	6.31	ns	5.73	5.86	ns	5.95	6.11	ns
pH	3.09	2.99	ns	2.94	3.08	ns	3.12	3.20	ns	3.04	3.01	ns	3.27	3.31	ns	3.22	3.09	ns

($p < 0.05$; A p-value less than 0.05 indicate statistical significance; SG- Single Guyot; DG – double Guyot; TSS – total soluble solids; TA – titratable acidity)

The p-values for grape yield at both 100% and 50% thinning percentages were greater than 0.05 for all growing seasons, indicating no statistically significant differences in grape yield between the SG and DG training systems throughout the study period. This suggests that neither training system yields consistently higher grape production over the years.

At a thinning percentage of 100%, significant differences were observed in bunches per vine between the SG and DG training systems in 2021 and 2022 ($p < 0.05$). Similarly, at a thinning percentage of 50%, significant differences were found in 2021 and 2022 ($p < 0.05$). However, no significant differences were observed in 2020 for either thinning percentage. This implies that the SG and DG training systems may influence bunch development differently in specific years, but not consistently across all years.

Significant differences in bunch weight between the SG and DG training systems were consistently observed for both thinning percentages across all three years ($p < 0.05$). This indicates that the choice of training system has a consistent impact on the weight of individual grape bunches with one system consistently producing heavier bunches than the other.

For all thinning percentages and years, the p-values exceeded 0.05, indicating no statistically significant differences in berry weight between the SG and DG training systems. This suggests that both training systems yield comparable berry sizes and do not significantly affect berry weight.

Furthermore, both TSS and TA levels displayed no statistically significant differences between the SG and DG training systems and thinning percentages across growing seasons, as indicated by p-values greater than 0.05. This suggests that the choice of training system does not significantly influence the sugar content (TSS) or acidity (TA) of the grapes.

Similar to TSS and TA, the p-values for pH levels exceeded 0.05 for all years and thinning percentages, suggesting no significant differences in pH between the SG and DG training systems. This indicates that both training systems result in grapes with similar pH levels, suggesting no significant impact on grape acidity.

The results indicate that there are statistically significant differences between the Single Guyot and Double Guyot training systems for the variables bunches/vine and bunch weight (g/bunch) in 2021 and 2022. However, for the other variables (grape yield, berry weight, TSS, TA, and pH), there are no statistically significant differences between the two training systems across all three years. This suggests that the choice of training system may have a significant impact on certain grapevine characteristics (Del Zozzo & Poni, 2024) specifically bunch-related variables, in certain years. Further investigation may be needed to understand the underlying factors contributing to these differences.

CONCLUSIONS

The results of the statistical analysis shed light on the significant influence of training systems on leaf characteristics within the context of Italian Riesling grape cultivation. Across all three years, notable disparities were observed in leaf area per vine, leaf weight per vine, and leaf area on the main shoot between the Single Guyot (SG) and Double Guyot (DG) systems. Consistently, SG exhibited higher values for these parameters compared to DG, underscoring the pivotal role of the chosen training system in shaping vine development and canopy management.

While there remained a stable level of variability within each training system over the study period, the differences between SG and DG systems remained statistically significant. This consistency implies that vineyard managers can rely on the predictability of each system's performance, aiding in informed decision-making for optimal vineyard management practices.

Moreover, the study elucidated that thinning practices, applied in 100% in both SG and DG systems, did not substantially alter the observed differences in leaf characteristics. This underscores the paramount importance of selecting the appropriate training system to achieve desired outcomes in vineyard management.

Additionally, investigations into harvest time and berry quality revealed no significant disparities in grape yield, berry weight, total soluble solids (TSS), titratable acidity (TA), and pH levels between SG and DG systems across the study duration. While training systems may influence leaf characteristics, their impact on grape yield and quality parameters appeared negligible.

Nonetheless, notable differences in bunches per vine and bunch weight between SG and DG systems were noted in specific growing seasons (2021 and 2022). This variability underscores the multifaceted nature of environmental factors and management practices influencing grapevine development and highlights the need for further research to elucidate underlying mechanisms.

The findings underscore the critical importance of selecting the most suitable training system for vineyards, with SG showing consistent advantages in leaf characteristics. However, the variability observed in bunch-related variables emphasizes the complexity of vineyard management and calls for tailored strategies based on specific conditions and goals.

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RESEARCH CONCERNING THE UTILISATION OF THE UNDERGROUND DRIP IRRIGATION IN ORDER TO CONTROL THE WATER STRESS OF GRAPEVINE

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Abstract

The research was carried out in the period 2019-2023 in an experimental polygon with Chardonnay and Sauvignon varieties, in the conditions of a reddish-brown molic soil and Feteasca regala variety under the conditions of an anthrosol hortic, argic, vertic. According to the level of the rainfalls that induced a certain level of soil humidity, four water supplies were yearly applied, the irrigation amount ranging in between the limits of 1100-2225 cubic meters/ha. Therefore, during the whole green period, the irigated variants were supplied with a water reserve in the soil exceeding the minimal threshold by 50% of the Useful water capacity (UC). The results obtained evidenced the positive influence of irrigation both upon the grape yield and its quality. Increased yields of grapes were obtained in comparison with the not irrigated witness control, averagely ranging in between the limits of 12 - 64%, on the background of a better accumulation of sugars in grapes. Under severe conditions of water stress, the application of irrigation proves to be compulsory, in certain years even after the beginning of the grape ripening stage.

Key words*: water stress, pedological drought, Active Humidity Index.*

INTRODUCTION

In the current context of climate change, which has led to the emergence of different periods of time (longer or shorter), with increased water stress, during the vegetative period, vine irrigation has become a necessity, even in vine-growing areas that two decades ago they were considered wet.

Although the vine easily adapts to drought conditions (having a well-developed root system in depth and with a high absorption capacity) being able to use the moisture from the soil up to values close to the wilting coefficient, the lack of water from the soil can induce critical periods from a physiological point of view, which will reflect not only on the yield of the current year, but also on future harvests.

In order to develop plants with normal vigor, able of producing sustained harvests each year, the vine needs large amounts of water. Thus, a vine bush consumes through transpiration during the vegetation period between 850-1200 l of water, which corresponds to a consumption of 3500-5000 m3/ha (depending on the plantation size) (Plesa et al., 1970; Canarache, 1990).

The highest water requirements are manifested during the period of intense shoot growth and fruit formation (reaching a maximum level in the phenophase of berry growth), until grapes enter the veraison phase. In this interval, consumption is on average around 22.8-23.3 m^3 water/day/ha (Goldhamer, 2005).

In Romania, the grapevine culture in a non-irrigated regime is possible only in the vine-growing areas, where an annual amount of precipitation between 400-700 mm is recorded, of which at least 250 mm evenly distributed during the vegetation period, as useful rainfall (greater than 10 mm) (Oşlobeanu et al., 1980; Grumeza, 1968; Deloire, 2008).

Considering the high water consumption, for normal growth and productivity, the vine requires an optimal soil moisture at the minimum treshhold (Tm), the lower values being favorable for the grape berries ripening, and the higher values for the shoots growth (Stewart, 2005).

A reduced consumption of water is recorded only in the period between the beginning of vine bleeding to bud burst and during the grape ripening period.

If during the autumn-winter period a high reserve of water from rain and snow accumulates in the soil, the vine does not show phenomena of water stress even in some drier summers. Conversely, if a dry autumn-spring period is followed by a dry summer, the effects of the drought become disastrous, going as far as drying out the plants.

MATERIALS AND METHODS

The study was carried out during the years 2019-2023, in the Valea Călugărească viticultural center, in two viticultural plantings with Chardonnay, Sauvignon and Fetească regală varieties.

The soil in the experimental polygon with the Chardonnay variety is reddish brown molic vertic, with a loam clay texture, weak acid pH (6.1), well supplied with humus (2.9%) and useful mineral elements (N, P, K). The soil in the experimental polygon with the Feteasca regala variety is an anthrosol hortic, argic, vertic, with a clay loam texture, weak alkaline pH (7.6) and low nutrient content N, P, K.

The vines were led in a semi-high form, in the form of a bilateral cordon, the cutting was carried out in short fruit elements (renewal spur), with a fruit load of 18 buds/m^2.

The climate data used in this study were provided by the Research Institute for Viticulture and Enology Valea Calugareasca station, for 2019-2023 period. The data consist of daily observations of the monthly average temperature and rainfall.

The mobility and accessibility of soil water was characterized by a series of hydrophysical indices: apparent density (DA); hygroscopicity coefficient (CH), field water capacity (CC), wilting coefficient (CO), usable water capacity (CU), minimum threshold (Tm) for soil moisture.

CO = 1.5* CH; CU=CC-CO

The minimum threshold (Tm) of soil moisture was obtained by using the calculation formula:

PM= CO+f*CU,

where f = the fraction of the accessible moisture range for which we use the value ½, value specific to the clay-loamy texture.

At the beginning of the vegetation period (April), the initial reserve of water in the soil was determined by performing humidity tests on collected soil samples.

Then monthly monitoring continued until the end of September, when the final soil water reserve was determined.

Based on the obtained results, the deficit or excess compared to the minimum threshold of 50% from the usable water capacity, was established.

When watering is applied, in conditions of humidity, different from the minimum threshold, we have to deal with norm of real watering (mr): mr=H*DA*(CC-w), where: H represents the soil layer thickness to be watered (m); DA-apparent density (t/mc); CC-field capacity (% w/w) and w-current soil water moisture (%w/w).

The vine hydration status was assessed also by measuring the leaf water potential in the Scholander pressure chamber (Scholander et al., 1965; Turner, 1988).

The measurements were made from the phenophase of grape berry development until harvest, in the morning, before sunrise, because the hydration state of the leaves is at the maximum level that the water in the soil can provide. (Carbonneau, 1996; Carbonneau et al., 2004; Ojeda et al., 2002).

Depending on the soil characteristics, the precipitation deficit, the vegetative phenophase, the soil water moisture and foliar water potential, the watering rate was established.

Based on the decade and monthly analysis of the soil water, the watering moments were established such that the water from the ground to be maintained above the minimum threshold.

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 experimental variants. The following analyses were performed: sugar content - determined by refractometry (OIV 2021a); total acidity - determined by titration with NaOH (OIV 2021b).

RESULTS AND DISCUSSIONS

The meteorological data recorded in the 2019-2023 period indicate a thermal regime characterized by average annual temperatures that oscillate between 11.8°C (2021) and 13.6°C (2023).

Compared with normal (1989-2018), the average temperature during the vegetation period increased by 0.6°C (2022), 0.8°C (2019), 0.9°C (2020), respectively 1.3°C (2023) (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, 36.0 mm. This became an excess in May when 84.5 mm was recorded compared with 71.6 mm, the multi-year average. (Figure 2).

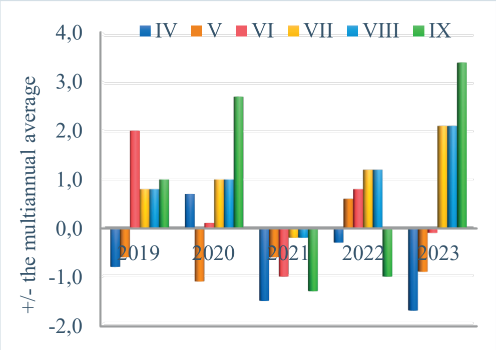

Figure 1. Monthly average temperature during the growing season of the years 2019-2023 compared to the multiannual average of 1989-2018

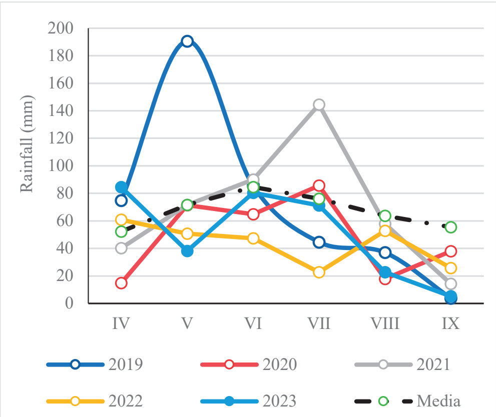

Figure 2. Monthly average rainfall during the growing season of the years 2019-2023 compared to the multiannual average of 1989-2018

The pluviometric characterization of the years 2019-2023 highlighted the fact that the precipitation deficit was recorded, in general, during years 2020, 2022 and 2023. Thus, two intervals with pluviometric deficit were highlighted: in the phenophases from bud burst to the intense growth of the shoots and fruit formation, and more intensively in the phenophases from the veraison to the grapes ripening.
The apparent soil density of the soils shows in the hoeing horizon (0-60 cm), an average values of 1.42 g/cm^3 (reddish brown molic soil), but also a high value (1.65 g/cm3) in the case of anthrosol hortic, argic, vertic.
The values of the main hydrophysical indices (Tables 1 and 2) are mainly influenced by soil texture.

Table 1. The physico characteristics of reddish, mollic, vertic soil (depth 0-100 cm)

Depth cm	Apparent density (DA) t/m^3	Wilting coefficient (CO) %	Field capacity (CC) %	Minumum threshold (Tm) %
0-20	1.38	11.2	23.6	17.4
20-40	1.42	11.4	23.9	17.7
40-60	1.45	11.5	24.5	18.0
60-80	1.46	11.9	24.9	18.4
80-100	1.50	11.2	25	18.1

Table 2. The physico characteristics of hortic, argic, vertic anthrosol (depth 0-100 cm)

Depth cm	Apparent density (DA) t/m^3	Wilting coefficient (CO) %	Field capacity (CC) %	Minumum threshold (Tm) %
0-20	1.69	9.8	20.0	14.9
20-40	1.64	9.9	20.1	15.0
40-60	1.61	9.9	20.2	15.1
60-80	1.60	10.0	20.5	15.3
80-100	1.65	10.2	20.9	15.6

Thus, the hygroscopicity coefficient (CH) registered values between 7.6%, for the mollic red brown soil, and 6.6%, for the hortic anthrosol.
A similar variation is presented by the wilting coefficient (CO) values, which are between 11.4% for the mollic reddish preluvosol and 9.9% for the hortic anthrosol.
The useful water capacity generally recorded values between 12.7% for the soft reddish-brown soil and 10.3% for the hortic, argic, vertical anthrosol soil.
Both soil types were able to store a useful water volume (CU) of 1829 m^3/ha (reddish-brown soil) and 1489 m^3/ha (vertical anthropogenic soil).
By analyzing the evolution of soil moisture at the depth of 0-100 cm during the vine vegetation period, it was found that at the beginning of the

vegetation period, April, the soil moisture was at a normal level.
Starting with July, in all the studied years, the soil water reserve values were below the minimum threshold (Tm) of 50% of the usable water capacity (2556 m^3/ha). This fact indicates the onset of soil drought and the need to apply irrigation in the Chardonnay vineyard (Figure 3).

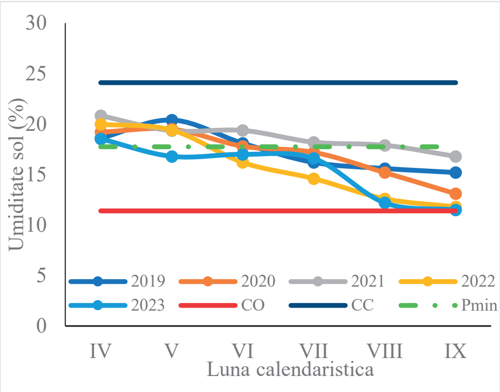

Figure 3. Dynamics of the soil water reserve in the Chardonnay plantation on the 0-100 cm profile

The soil moisture variations during the vegetation period of the Feteasca regala variety, on the 0-100 cm profile, oscillated between 12.3% (2023) and 13.7% (2020) in the phenophase of grapes veraison.
Soil moisture was maintained below the minimum threshold level of 15.2% and in the phonophase of grape ripening with variations between 10.5% (2023), 11.2% (2020) and 12.4% (2022) (Figure 4).

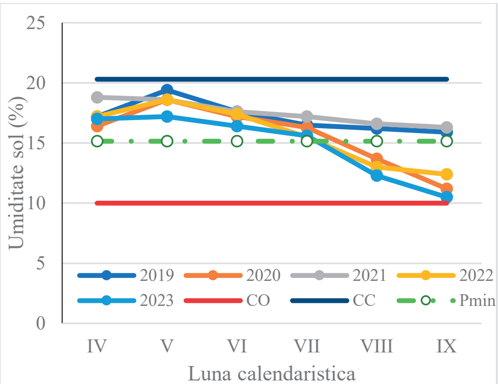

Figure 4. Dynamics of the soil water reserve in the Feteasca regala plantation on the 0-100 cm profile

The occurance of accentuated water stress periods, longer or shorter, during the vegetation phase of the vine required the application of irrigation norms with the provision of water at the minimum threshold level. In order to maintain the soil water reserve at the level of the minimum threshold in the Chardonnay plantation, in the irrigated variants, 3 waterings were carried out, with irrigation norms between 1836 m^3/ha (2020) and 2225 m^3/ha (2023), applied in the period of intense growth of berries until the grape ripening (Table 3).

Table 3. Water regime in the soil during the vegetation period of Chardonnay variety (depth 0-100 cm)

Variant	Initial reserve 1 IV		Norm of irrigation	Final reserve 30 IX	
	m^3/ha	Surplus to Tm m^3/ha		m^3/ha	Difference from Tm m^3/ha
Year 2019					
Non-irrigated	2621	65	-	2102	-454.0
Irrigated	2678	122	1102	2189	+202.0
Year 2020					
Non-irrigated	2419	-137	-	2004	-821
Irrigated	2765	209	1836	2844	+288
Year 2021					
Non-irrigated	2822	326	-	1930	- 626
Irrigated	3000	444	698	2901	+ 482
Year 2022					
Non-irrigated	2722	166	-	1858	- 864
Irrigated	2877	321	2196	2700	+ 144
Year 2023					
Non-irrigated	2592	36	-	1771	-785
Irrigated	2688	112	2225	2772	+ 216

In the non-irrigated variant (control), the water deficit from the soil manifested during the vine vegetation period in all the studied years.
The difference compared to the minimum threshold was - 454 m^3/ha (2019) to - 864 m^3/ha (2022).
In the Feteasca regala plantation, the soil water reserve at the minimum threshold level was maintained by applying irrigation norms of 804 m^3/ha (2022), 886 m^3/ha (2020) and 1230 m^3/ha (2023), in the periods with water stress, from the veraison to the grape ripening (Table 4).
In the variant of the non-irrigated varieties, the decrease of soil moisture at the level of the withering coefficient, during the vegetation period, determined a stoppage of vegetative growth with a strong impact on grape production and its quality, by reducing the process of sugars accumulation in the grape

berries. The degree of stress (leaf water potential or LWP) was measured by using the Scholander pressure chamber (Scholander et al., 1965).

Table 4. Water regime in the soil during the vegetation period of Feteasca regala variety (depth 0-100 cm)

Variant	Initial reserve 1 IV		Norm of irrigation	Final reserve 30 IX	
	m^3/ha	Surplus to Tm m^3/ha	m^3/ha	m^3/ha	m^3/ha
Year 2019					
Non-irrigated	2434	252	-	2088	-94.0
Irrigated	2477	295	-	2290	+108.0
Year 2020					
Non-irrigated	2304	122	-	2016	-166
Irrigated	2362	180	886	2499	+317
Year 2021					
Non-irrigated	2477	295	-	2045	- 137
Irrigated	2707	526	-	2347	+ 166
Year 2022					
Non-irrigated	2434	252	-	1757	- 425
Irrigated	2477	295	804	2589	+ 407
Year 2023					
Non-irrigated	2347	166	-	1613	-569
Irrigated	2448	266	1230	2742	+ 560

The pressure chamber, indicates the value of the pressure exerted on the leaf petiole in bars (Carbonneau et al., 2004). The degree of stress for Chardonnay variety was moderate at 12-14 bars (2020, 2022, 2023) in the veraison phenophase, and severe with values of 16.6-17.2 bars (2022, 2023), at the ripening of the grapes.The water availability in the soil layer, explored by the roots, in the plantation of Feteasca regala variety indicates a high stress at the veraison phenophase of grapes, with values from 14.6 bars (2022) to 15.9 bars (2023), and when grapes matured the stress increased, taking over values from 16.2 bar (2021) to 16.6 bar (2022) (Table 5).

Table 5. Water potential of vines (bars)

Year/Month	IV	V	VI	VII	VIII	IX
Chardonnay						
2019	3.6	2.4	4.8	**10.4**	12.8	15.6
2020	4.2	2.8	6.4	**11.9**	14.6	**15.9**
2021	2.6	3.2	5.9	10.7	11.7	**16.2**
2022	3.1	3.6	6.3	**12.8**	12.9	**16.6**
2023	3.7	4.1	7.2	**14.4**	**13.3**	**17.2**
Feteasca regala						
2019	2.1	2.6	3.4	2.7	7.3	7.5
2020	3.6	2.1	3.5	2.6	**15.4**	**16.2**
2021	3.8	2.6	2.7	3.2	6.8	8.4
2022	2.8	3.1	1.8	4.2	**14.6**	**15.8**
2023	3.4	3.5	3.8	5.4	**15.9**	**16.4**

The positive effect of irrigation was more pronounced on the grape yield t/ha and on the average weight of a grape, a fact which sustained, in the irrigated variants, yield increases between 30-65% on average, compared to the non-irrigated control and a weight average of a grape 26-37% (Table 6).

Regarding the quality of production for Chardonnay grapes, expressed by the sugar content and the total acidity of the must, it should be noted that in the case of the irrigated variants, sugar content increased between 7.0 (2019) and 23% (2023), on the background of a relatively constant must acidity, with decreased of 5.0 (2019) and 9.0% (2023).

Table 6. Influence of irrigation on quantitative and qualitative production of Chardonnay grapes

Variant	Yield t/ha	Average weight of a grape, g	Sugar g/l	Acidity g/l H_2SO_4
Year 2019				
Non-irrigated	6.8	68.6	182.7	3.4
Irrigated	8.8	86.4	195.5	3.2
Year 2020				
Non-irrigated	5.4	72.4	194.5	3.6
Irrigated	7.5	95.6	217.8	3.3
Year 2021				
Non-irrigated	7.2	75.8	187.4	4.3
Irrigated	10.9	101.6	219.3	4.0
Year 2022				
Non-irrigated	8	77.2	196.7	3.6
Irrigated	13.1	105.8	232.1	3.4
Year 2023				
Non-irrigated	8	78.9	192.7	3.5
Irrigated	13.2	106.5	237.0	3.2

In case of Feteasca regala variety, under conditions of the years 2020, 2022 and 2023, when at the end of the vegetation period the deficit of water from the soil, in the non-irrigated variant, was approximately 166 m^3/ha (2020), 425 m^3/ha (2022), 569 m^3/ha (2023).

Yield increases between 56-64% were achieved through irrigation with norms between 886 m^3/ha (2020), 804 m^3/ha (2022) and 1230 m^3/ha (2023) were between, 8-21% sugar content and constant acidity (Table 7).

Table 7. The influence of irrigation on the quantitative and qualitative production of Feteasca regala grapes

Variant	Productively t/ha	Average weight of a grape, g	Sugar g/l	Acidity g/l H2SO4
Year 2019				
Non-irrigated	8.6	88.9	190.6	3.9
Irrigated	8.9	94.2	200.1	3.8
Year 2020				
Non-irrigated	8.5	95.7	192.4	4.2
Irrigated	12.1	111.0	215.5	3.9
Year 2021				
Non-irrigated	8.2	86.5	188	4
Irrigated	9.2	98.6	203.0	3.8
Year 2022				
Non-irrigated	6.8	97	186.7	4.6
Irrigated	10.6	128.0	212.8	4.2
Year 2023				
Non-irrigated	7	102	190	4.2
Irrigated	11.5	134.6	229.9	3.9

CONCLUSIONS

The obtained results highlighted the positive effect of irrigation on grape production and its quality.

Through irrigation with input rates between 1836-2225 m^3/ha, for Chardonnay variety (depending on humidity conditions) production increased with 30-65% and a better accumulation of sugars in grapes by 12-23% was recorded. The medium weight of a grape bunch registered increases ranged between 26-35%.

Due to the clayey texture of the anthrosol hortic, argic, vertic, the irrigation norms are reduced, because the water retention capacity was higher, ensuring increases of production.

Regarding the Feteasca regala variety, by applying the irrigation norms of 886-1230 m^3/ha, production increases of 12-64% were achieved, with a grape weight of 14-32% higher and an increased sugar content in must with 8-21%.

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EFFECT OF BUNCH THINNING ON THE GRAPES QUALITY IN CLONES 174 AND 470 FROM SYRAH VARIETY

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Abstract

The experiment was conducted in the experimental vineyard of the Agricultural University - Plovdiv, during the period 2020-2022. Two clones of the Syrah variety numbered 174 and 470 grafted onto the SO4 rootstock were used. The green pruning operation `bunch thinning` was applied, performed manually in the `pea size` phase. Differences were found in the bunch structure according to the indicators - normal berries (%), bunches (%), milleranded berries (%), raisined berries (%), theoretical yield (%), average bunch mass (g) and average cluster size (cm). The mechanical analysis was obtained from skins (%), seeds (%), mesocarp (%), average weight of 100 berries (g), number of seeds in 100 berries, average seeds weight in 100 berries (g), average berry size (mm). The grape ripening dynamics was monitored, through the quantitative change in the sugar content (%) and titratable acids (g/l). The application of bunch thinning, as a widely known viticultural practice, part of the Canopy management complex of activities involved in, has had a positive impact on accelerating the grapes ripening and increasing its quality in the studied clones.

***Key words**: bunch thinning, clones, mechanical analysis, Syrah variety, technological maturity.*

INTRODUCTION

Climate changes require adaptation to higher temperatures, which includes changes of plant material (rootstocks, cultivars and clones) and modification of viticultural techniques (change in stem height, ratio of fruit weight to plant mass, time to carry out the pruning) so that the grape harvest takes place in the optimal period until the end of September or the beginning of October in the Northern Hemisphere (Van Leeuwen et al., 2019). The substrate has a significant influence on the quantity and quality of grapes, which is determined by the growth vigor, the different metabolism of the nutritional elements and compatibility with the variety, as well as in stress conditions caused by drought (Kocsis L. et al., 2012; Yoncheva T. et al., 2022). Moderate water stress during the veraison is favorable for sugar accumulation and also leads to an increase in the content of anthocyanins and tannins in the fruit (Van Leeuwen et al., 2009).

Increased climate instability (Webb et al., 2012), the implementation of sustainable management practices (Daane et al., 2018) and increasement in the price of raw materials are a test of sustainability in the development of viticulture sector. Due to the complexity of vine physiology, the effect of these practices on productivity can vary widely and results in yield parameters and fruit composition are varietal and location dependent. (Palliotti et al., 2014).

Bunch thinning is a widespread technological practice, improving grape quality depending on time and intensity of its application. The effect on Syrah is more noticeable in wine, but in order to make a decision and justify its price, a significant increase in the fruit quality must be achieved (De Bei et al., 2022).

Many studies have reported yield reductions in response to thinning at flowering stage (Reynolds et al., 1994), pea size stage (Keller et al., 2005; Sun et al., 2012) and during ripening (Keller et al., 2005; Gil et al., 2013). However, yield loss is not proportional to thinning intensity due to compensation, through increased berry and bunch weight (Reynolds et al., 2007; Sun et al., 2012; Gil et al., 2013).

Regina et al. (2005), found that clone 470 grafted on SO4 was the earliest and had the potential to produce grapes of good quality but with lowest productivity among the other clones.

Berry size is a quality factor directly related to grape thinning. The physical characteristics such as mass, volume and skin area increased with increasing size, showing a uniform trend.

Positive correlations were found between berry weight, volume and skin area, and these variables were negatively related to the number of berries in kilograms of grapes. Fruit volume is negatively correlated with dry skin weight. Skin area versus fruit volume is an indicator of the thinning effect associated with increasing size, as larger fruits have lower values (Melo et al., 2015).

Overloading the vines with grapes is the reason for incomplete shoots ripening and the insufficient supply of plastic substances (Popova, 2021). Recent research has revealed that grape thinning can increase sugar content and cause further changes to the grape composition (Wang Wen et al., 2022). Bunch thinning is an alternative method to improve ripening of wine grapes (Gil et al., 2013).

The purpose of the present study is to show the difference (deviation) in the ripening and grape quality, as a result of yield reduction in berries pea-size phase in clones 174 and 470 from Syrah variety, planted in the area of Brestnik village.

MATERIALS AND METHODS

For the purpose of the study, clones from Syrah variety numbered 174 and 470 were used, grafted onto Berlandieri x Riparia SO4 rootstock, planted in April 2011 at the Educational, Experimental and Implementation Base of the Department of Viticulture and Fruit Growing at the Agrarian University - Plovdiv, located in the land of the Kuklen town and the village of Brestnik, Rodopi municipality.

The vineyard is in full fruition. The planting distance is 3.0 m between the rows and 1.00 m between the vines in the row - 3330 vines per hectare. Vines are grown on high-stemmed. The training system is a double-sided cordon with the corresponding support trellis. The load in all variants was carried out by pruning spurs with two buds each, a total of 6 spurs/12 buds/ per vine. The inter-rows are grass covered, the soil surface between the vines is kept clean by treatment with herbicides.

Experimental scheme includes the following 4 options:

V1 - Syrah variety, clone 174 - non-reduced yield

V2 - Syrah variety, clone 470 - non-reduced yield;

V3 - Syrah variety, clone 174 - reduced yield;

V4 - Syrah variety, clone 470 - reduced yield.

Each variant includes 60 vines (4 repetitions x 15 vines).

The yield rationing was carried out during the vegetation in the "pea size" phase, leaving 8 bunches per vine.

The study includes indicators characterizing and describing the grape quality:

• Tracking the dynamics of sugars, with Abbe's laboratory refractometer, %.

• Tracking the change of titratable acids, by titration with 0.1 n NaOH, g/dm^3. The determination of sugars and titratable acids were studied from the beginning of veraison in order to establish the period of technological maturity (grape harvest), according to the methodology described in the Manual for Exercises in Winemaking (Bambalov, 2009).

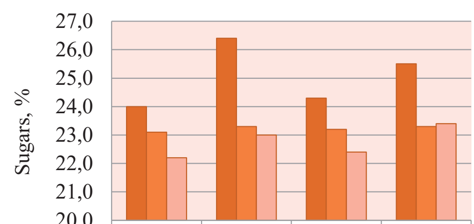
• Mechanical structure of bunch and berry: normal berries (%), bunches (%), skins (%), seeds (%), milleranded berries (%), mesocarp (%), theoretical yield (%), average bunch size - length and width (cm), average berry dimensions - length and width (mm), chemical composition of grape juice - sugars (%) and titratable acids (g/l). The mechanical analysis of grapes and berries in the studied clones was carried out with average samples of about 3 kg of grapes per variant, on the harvest day.

The indicators for the mechanical composition of the grapes were studied according to the generally accepted methodology described in the Students' guide to Ampelography (Roychev, 2014).

The obtained data were mathematically processed by the method of variance analysis using the SPSS program, and to establish the differences between the investigated variants, Duncan's multirank test was used, with the smallest significant difference (LSD) - 0.05 (5%).

RESULTS AND DISCUSSIONS

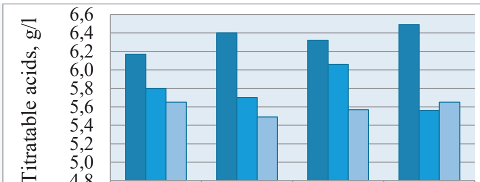
The grape quality for wine varieties depends mainly on the content of sugars and titratable acids, which serve to determine the technological ripeness. During the ripening period, which in phenological terms is from the beginning of ripening to grape harvest, the dynamics of sugar and acid content in the grapes was followed. During three experimental years, that is the period from the last ten days of July to the onset of technological maturity (beginning of September). The dynamics of sugars in clones included (Figure 1) proceeds smoothly without sharp fluctuations. Clone 470 is distinguished by a higher sugar accumulation, both in the vines with non-reduced yield (V2 - 26.4%, 23.3% and 23.0%), and in the vines with reduced yield (V4 - 25.5, 23, 3% and 23.4%).



	Clone 174 -V1	Clone 470 -V2	Clone 174 -V3	Clone 470 -V4
2020	24,0	26,4	24,3	25,5
2021	23,1	23,3	23,2	23,3
2022	22,2	23,0	22,4	23,4

Figure 1. Dynamics of sugar accumulation, %

Changes in titratable acidity is inversely proportional to sugars (Figure 2), which gradually decrease during grape ripening, with their values remaining slightly above the limits for quality red wines of 5.5-6.4 g/l.



	Clone 174 -V1	Clone 470 -V2	Clone 174 -V3	Clone 470 -V4
2020	6,2	6,4	6,3	6,5
2021	5,8	5,7	6,1	5,6
2022	5,7	5,5	5,6	5,7

Figure 2. Dynamics of titratable acids accumulation, g/l

Titratable acids were relatively high in the first experimental year (2020), when a higher sugar content was also found. Technological maturity for variants with reduced yield occurs on 2nd of September, and for non-reduced vines on 9th September, 2020.
In the second experimental year, the harvest was carried out on August 30, for the variants with reduced yield (V3 and V4), and on September 7, 2021, for the variants with non-reduced yield (V1 and V2). In the third experimental year, the grape harvest of the vines with reduced yield was carried out on August 30, and for the vines with non-reduced yield on September 8, 2022.
On the harvest day, the average values of sugar in the clones with non-reduced (V1 and V2) and reduced yield (V3 and V4) were 23.6%, and the titratable acid content 5.8-5.9 g/l, which is an indicator, that grapes from three harvests were harvested when the same values of the main components were reached. During the research period, when determining grape harvest date, together with the chemical composition of the grapes, we took into account both its sanitary condition and climatic conditions. From the complex assessment of these indicators, it was found that the variants with reduced yield (V3 and V4) managed to accumulate sugars faster, compared to the non-reduced variants (V1 and V2), which is indicative of the earlier onset of technological maturity in the three harvests.
For more detailed study on quality, a complete mechanical analysis was carried out, which expresses the ratio between the elements of grapes and berries - bunches, skins, mesocarp (fleshy part) and seeds, determined by weight, by their mass and number. The ratio between the bunch mass and berries, as well as the individual berry parts, in percentages relative to the total bunch mass, reflecting objective digital data, the correct sampling of average grapes is important (Popova, 2023). The test results are presented in detail in Tables 1, 2, 3 and 4, being determined on the average basis samples collected during the grape harvest.
The percentage of normally developed berries for all variants was high from 94% to 96%, which is a prerequisite for very good grape quality (Tables 1 and 2). There are proven mathematical differences between the individual experimental variants in the vines with reduced yield in clone 174 (V3) and 470 (V4). The percentage of berries for 2020 is from 4.20% (V1) to 5.55% (V3), in 2021 it is from 3.76% (V2) to 5.60% (V1) and from 3.08 (V4) to 4.39% (V3) for 2022.
The percentage of milleranded berries is small, with the largest proven difference in clone 470 (V4) in 2020. When summarizing the results, it

was found that the average mass per bunch was significantly greater in the vines with reduced yield, with the most significant difference in 2022 at clone 470 (V4). Regarding the indicator of average bunch size, the trend for larger bunches is confirmed in the variants with reduced yield (V3 and V4). The theoretical yield is high in all three years, with no significant differences between variants.

Table 1. Cluster structure and construction in non-reduced variants, for the period 2020-2022

Exp. Variants/ Clone	Period (Year)	Normal berries,%	Skins,%	Millerended berries,%	Dry berries,%	Theoretical mass,%	Average bunch weight, g	Average bunch size, cm	
								Length	Width
V_1 174	2020	95.80a	4.20a	0.47b	0.94b	80.70b	136.00b	19.20b	8.40a
	2021	94.40a	5.60c	0.59a	1.05b	79.14b	116.00ab	18.47a	10.35a
	2022	95.67a	4.33ab	0.18b	0.45a	80.57a	167.00b	19.45b	9.75a
	Average	**95.29**	**4.71**	**0.41**	**0.81**	**80.14**	**139.67**	**19.04**	**9.50**
V_2 470	2020	94.80a	5.12c	0.35a	0.81a	77.45a	142.00c	19.00b	8.80a
	2021	96.24a	3.76a	0.16a	0.99b	79.58ab	133.00c	20.50b	10.25a
	2022	96.28a	3.72a	0.12a	0.79b	80.88a	195.00c	19.50b	9.85a
	Average	**95.77**	**4.20**	**0.21**	**0.86**	**79.30**	**156.67**	**19.67**	**9.63**
LSD 5%	**2020**	**4.89**	**0.37**	**0.02**	**0.10**	**3.01**	**5.87**	**1.68**	**0.55**
LSD 5%	**2021**	**4.16**	**0.84**	**0.06**	**0.11**	**2.01**	**8.09**	**2.31**	**0.99**
LSD 5%	**2022**	**5.12**	**0.81**	**0.05**	**0.15**	**2.89**	**10.02**	**1.20**	**0.80**

*Comparative analysis with provenance α = 0.05.

Table 2. Cluster structure and construction in reduced variants, for the period 2020-2022

Exp. Variants/ Clone	Period (Year)	Normal berries, %	Skins, %	Millerended berries, %	Dry berries, %	Theoretical mass, %	Average bunch weight, g	Average bunch size, cm	
								Length	Width
V_3 174	2020	94.45b	5.55b	0.43b	0.54b	79.78b	172.00d	22.10b	11.10b
	2021	95.19ab	4.81b	0.19a	0.52b	80.50a	154.00 b	20.05a	10.50a
	2022	95.61b	4.39c	0.13a	0.49a	80.96a	228.00b	21.90b	11.45b
	Average	**95.08**	**4.92**	**0.25**	**0.52**	**80.41**	**184.67**	**21.35**	**11.02**
V_4 470	2020	95.33b	4.67a	0.57c	0.55b	77.76a	166.00c	22.10b	10.60a
	2021	95.67b	4.33a	0.16a	0.72b	79.55a	155.00b	22.05c	10.60a
	2022	96.92c	3.08a	0.46b	0.10b	81.78a	276.00d	21.85b	10.95a
	Average	**95.97**	**4.03**	**0.40**	**0.46**	**79.70**	**199.00**	**22.00**	**10.72**
LSD 5%	**2020**	**1.35**	**0.51**	**0.15**	**0.26**	**2.02**	**5.87**	**0.50**	**0.55**
LSD 5%	**2021**	**1.07**	**0.31**	**0.17**	**0.18**	**2.48**	**4.18**	**0.36**	**0.73**
LSD 5%	**2022**	**1.05**	**0.48**	**0.20**	**0.28**	**2.50**	**5.21**	**0.55**	**0.60**

*Comparative analysis with provenance α = 0.05.

The indicators for bunch structure and construction (Tables 3 and 4) are determined by a sample of 100 berries in typical shape and size. It was selected in advance by counting three samples of 100 berries, weighed separately, and the average (intermediate) value sample was used (Popova, 2023).
The skin mass in three years period is from 11.15% (V3) in 2022 to 14.76% (V2) in 2021. The biggest difference was found in clone 470 (V4) during 2022. The seed percentage ranged from 3.19 (V3) to 3.99 (V4), where the greatest statistical difference was found, while in the mesocarp, no significant difference was observed.
The mass of 100 berries and the mass of seeds in 100 berries is higher in the variants with reduced yield (V3 and V4) compared to those with non-reduced yield (V1, V2). The data for average berry size (length/width) show a greater difference in the vines with reduced yield, the most significant difference being in the variant V3 (Tables 3 and 4).

Table 3. Berry structure and construction in non-reduced variants, for the period 2020-2022

Exp. Variants/ Clone	Period (Year)	Skins, %	Seeds, %	Mesocarp, %	Average weight per 100 berries, g	Number of seeds in 100 berries	Average seeds weight in 100 berries, g	Average berry size, mm	
								Length	Width
V_1 174	2020	12.26a	3.47b	84.27a	159.86b	205a	5.55b	14.40b	13.70b
	2021	12.89ab	3.27a	83.84a	132.29a	180b	5.65b	13.50a	11.80a
	2022	11.89a	3.89b	84.22a	177.8a	260b	6.90b	15.20a	13.50a
	Average	**12.35**	**3.54**	**84.11**	**156.65**	**215.00**	**6.03**	**14.37**	**13.00**
V_2 470	2020	13.42ab	3.54b	81.70a	151.03a	200a	5.35a	13.80a	12.60a
	2021	14.76b	3.89b	82.69a	136.70ab	225c	6.90c	13.40a	11.75a
	2022	12.21a	3.78b	84.01a	190.30b	245b	7.20c	15.70a	13.55a
	Average	**13.46**	**3.74**	**82.80**	**159.34**	**223.33**	**6.48**	**14.30**	**12.63**
LSD 5%	2020	3.05	0.35	7.13	5.18	30.13	0.23	0.57	0.77
LSD 5%	2021	2.66	0.36	8.26	8.41	20.77	0.36	0.75	0.60
LSD 5%	2022	2.81	0.34	5.29	6.27	20.01	0.39	0.67	0.69

*Comparative analysis with provenance $\alpha = 0.05$.

Table 4. Berry structure and construction in reduced variants, for the period 2020-2022

Exp. Variants/ Clone	Period (Year)	Skins, %	Seeds, %	Mesocarp, %	Average weight per 100 berries, g	Number of seeds in 100 berries	Average seeds weight in 100 berries, g	Average berry size, mm	
								Length	Width
V_3 174	2020	12.34b	3.19a	84.47b	155.10b	160a	4.95a	13,80a	11,80b
	2021	12.09a	3.34a	84.57b	149.70b	190a	5.90ab	14,35ab	12,35ab
	2022	11.15b	4.17c	84.68a	195.60a	285c	8.15c	15,60b	13,85a
	Average	**11.86**	**3.57**	**84.57**	**166.80**	**211.67**	**6,34**	**14,58**	**12,67**
V_4 470	2020	14.85c	3.58b	81.57a	142.33a	175b	5,10b	13,80a	11,40a
	2021	12.86b	3.99d	83.15a	146.50ab	215b	6,60c	14,00a	12,26a
	2022	12.18d	3.65b	84.17a	198.30a	235b	7,25b	14,10a	13,95a
	Average	**13.30**	**3.74**	**82.96**	**162.37**	**208.33**	**6,32**	**13,97**	**12,54**
LSD 5%	2020	0.74	0.15	1.29	6.42	15.24	0,18	0,59	0,31
LSD 5%	2021	0.67	0.15	1.25	7.10	18.62	0,27	0,68	0,32
LSD 5%	2022	0.71	0.21	1.42	7.20	19.02	0,42	0,72	0,42

* Comparative analysis with provenance $\alpha=0,05$

CONCLUSIONS

The studied Syrah clones are distinguished by a high percentage of normally developed berries over 95%, which is an indicator of good grape quality. Average mass per bunch was greater in the reduced yield variants, with weight ranging in clone 174 (V3) to clone 470 (V4). The same correlation was found for average bunch size, with the largest bunches being on clone 470 (V4). Reduced yield varieties have a lower percentage of skins and a higher percentage of mesocarp, which proves that an increase in berry size leads to a decrease in the ratio of skins to flesh.

Reduction in the number of bunches in the "pea size" phase has a favorable effect on the components for determining the technological maturity of grapes - the dynamics of sugar content and titratable acids. Within the experimental period, all variants reached the technological maturity stage early (beginning of September) for the studied area.

The grape harvest of vines with reduced yield (V3, V4) precedes those with non-reduced yield (V1, V2) by approximately one week.

Crop yield reduction during the growing season is a useful agriculture practice to increase grape size and improve overall grape quality.

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STUDY ON THE QUALITATIVE PROFILE OF SOME SEEDLESS VINE VARIETIES IN A TEMPERATE CLIMATE

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Abstract

In grapevine varieties - the lack of seeds seen from the perspective of two phenomena parthenocarpy and stenospermocarpy - has a major impact on the consumer, representing an important added economic value, currently being one of the most appreciated features of table grape varieties. In the present work, four varieties of grapes intended for fresh consumption but also for raisins were studied - 'Sultanina' (Thompson seedlles), 'Sublima', 'Supernova' and 'Călina'. The obtained results showed that the productive and qualitative performances obtained, expressed by the values of the productive indices and the organoleptic qualities (brix, glucose, fructose, acidity, gluco-acidometric index), both in the fresh product and in the raisins, correspond to the quality standards. The surprising fact is that these varieties ensure a double use - firstly as table grapes for fresh consumption and secondarily as raw material for obtaining raisins (artificial dehydration at a temperature of 50^{0}C).

Key words*: grape, raisins, temperature, varieties, yield.*

INTRODUCTION

Grapes (*Vitis vinifera*) are one of the most famous fruits in the world (Khiari et al., 2019; Olmo-Cunillera et al., 2019; Keqin Chen et al., 2022). In grapevine varieties - the lack of seeds seen from the perspective of two phenomena parthenocarpy and stenospermocarpy - has a major impact on the consumer, representing an important added nutraceutical and economic value, being one of the most appreciated features of table grape varieties along with the other qualities, like: table grapes are delicacy fruits, they are the requested and consumed fruit-food, due to the rich and varied chemical composition necessary for the health and vitality of the human body. Their consumption, both fresh, but also as a raw material in the confectionery industry, has always been an important economic pole. Regular and correct consumption in the fresh state has also proven its therapeutic action, given by the concentration of phenolic compounds (over 500 compounds) accumulated in the skin, pulp and seeds of a series of microelements that favor metabolism, the presence of resveratrol - a powerful antioxidant, a real inhibitor of cancer cell growth (Cichi et al., 2023; Stroe and Catuneanu, 2022; Mahanna et al., 2019; Tahereh et al., 2020; Benbouguerra et al., 2021), and last but not least, the presence of substances that favor the weight loss process, however they also contain glucose (Haitao et al., 2021). The high content of raisins in sugars, fiber, vitamins, minerals and antioxidants against the background of a low to moderate glycemic index helps to maintain the best oral pH and oral health. Therefore, raisins are usually consumed as healthy snacks or food additives (Khiari et al., 2019). Three-four decades ago, only varieties intended for obtaining raisins were cultivated - raisins are produced by partially dehydrating berries grape - but nowadays, in the world, the cultivation of these varieties has come to be practiced in over 30 countries, although for a long time their cultivation it could not exceed the limits of the warm climate (Mediterranean climate), where drying was done right under the open sky. In this context, obtaining seedless varieties has become a main objective in the breeding programs of countries with a wine-growing tradition, along with the increase in consumer demands and demands for seedless fruits and vegetables. It's weel known that in the raisin industry the varieties belonging to the 'Corinth' and 'Sultanine' sortogroups are the most used, but due to the fact that through their culture in more northern areas (they are of southern origin) they have a weak differentiation of the fruit buds, found in a small percentage of fertility buds, it was necessary to create and cultivate new varieties, varieties adaptable to less favorable climatic conditions (temperate climate). In this context, producers and researchers focus more on the quality of the product - described by the specific and also measurable carpometric indicators: berry size, shape, compactness of the grapes, color of the berries, consistency, taste and aroma of the pulp, thickness of skins, thickness of the skin layer, color uniformity at full maturity, ripening period or any other qualitative attribute specific to the fruit. Therefore, the assortment of new varieties introduced into the culture took on a new allure, that of ensuring a double use - first of all as table grapes for fresh consumption and secondarily as raw material for obtaining raisins. Against this background, the consumer market both globally and nationally has grown significantly from year to year (FAO, 2020), drawing attention to the need to ensure competitive products in terms of sensory and visual characteristics. In our country, table grapes currently have a share of only 6.9% of Romania's wine production (O.I.V., 2022), and on a global level, table grapes represent 31.5 million tons - 44.5% of the total wine production and dried grapes is 5.7 million tons - 8.0%. In other words, in Romania, the production of table grapes is almost stagnant in the last 10 years and at a quarter of the maximums recorded in the last 30 years.Based on these principles, the present study was approached starting from two considerations: to evaluate the quantitative and qualitative performances of the varieties in an area different from the one of origin and to appreciate their profile in terms of the possibility of obtaining raisins following the kinetics of individual ripening of the berries at the time of harvesting (quantitative evaluation of the fresh mass and sugar accumulation), related in the second plan to the dehydration capacity.

MATERIALS AND METHODS

Plant Material and Growth Condition

The research was carried out in the 2022-2023 seasons and for this were taken into study, 4 varieties of grapes intended for fresh consumption but also for raisins: 'Sultanina' (Thompson seeds), 'Sublima', 'Supernova' and 'Călina'. The first three varieties are some of the most cultivated in the world, but also in our country 'Sultanina' (Thompson seedlles) and a local variety Calina.The varieties are 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 www.vivc.de (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m). The experimental field 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 crop load distributed on the plant was 30 buds/vine. The variety 'Călina' was grafted on Teleki 4 SO4-4 Blaj (*Vitis berlandieri* × *Vitis riparia*) rootstock; The variety 'Sublima' and 'Supernova' were grafted on SO4-5 and variety 'Sultanina' was grafted on SO4-102. During the vegetation period, a whole set of green works and operations specific to the apyrene varieties was applied - the shoots were manually directed vertically whenever necessary and in the last days of July a slight shortening of the shoots was carried out, leaving a canpy eight of approximately 1.8 m. The vine was managed with irrigation in the critical phenophases (intense berrie growth) and a standard disease control program to control downy mildew, powdery mildew and gray rot (*Botrytis*), as well as applying a 23.05.2023 one treatment to stimulate the flower set process and balanced nutrition, thus increasing plants resistance to stress conditions as well as "hidden" deficiencies of other micronutrients. (quite difficult in seedless varieties).

Short presentation of variety 'Călina'

The variety studied was the Călina variety - It is the first apiren variety obtained in our country and immediately introduced into culture. a new apirin variety obtained in Romania in 1985 in the Research and Development Station for Viticulture and Oenology Dragasani-Valcea by Mircea Mărculescu in the cross section of the two varieties 'Braghina' and 'Sultanina'. The main direction of production is for fresh consumption, but also for the production of raisins. In terms of the appearance, it resembles the 'Sultanina' a variety.
https://www.vivc.de/index.php?r=passport%2Fview&id=1996

Short presentation of 'Sublima'

It is a variety native to Argentina, created by Angel A. Gargiulo at the National Institute of Agricultural Technology Rama Caida in Argentina. The parents of this variety are 'Carina' and Gargiulo 88435 ('Almeria' x 'Cardinal'). The main direction of production is for fresh consumption and production of raisins. https://www.vivc.de/index.php?r=passport%2Fview&id=17812

Short presentation of 'Supernova'

It was obtained by M.S. Zhuravel, G.M. Borzikova and I.P. Gavrilov at the Horticultural Research Institute of Uzbekistan. The parents are 'Cardinal' and 'Kişmiş Rozovyi'. The variety has a medium-large, ovoid, pink berrie. The flesh is crunchy and it is used in the country of origin both as a table variety and as a variety for raisins (https://www.vivc.de/index.php?r=passport%2Fview&id=15517)

Short presentation of 'Sultanina' (Thompson seedles)

It is a variety of Asian origin, the exact region of origin not being known. It is assumed that it originates from Iran or Turkey and is part of Proles orientalis - subproles antasiatica. The grapes of this variety are used especially for raisins and for consumption in the state fresh, juice (https://www.vivc.de/index.php?r=passport%2Fview&id=12051)

Climate Data

For this study, there were used weather data recorded at Bucharest-Baneasa meteorological station for the experimental period (2022-2023), a series of climatic data were studied (Table 1), and monthly average temperatures were used to evaluate a set of bioclimatic indices commonly used in viticulture: Huglin index (HI), Winkler index (WI) and cool night index (CNI).

Sampling and Chemical Analysis

In order to assess the descriptive parameters of the quality, determinations of biochemical parameters of the grapes was made, as follows: For the determination of fruit firmness, the electronic penetrometer TR was used, with a piston of 3 mm diameter, the results being expressed in N/cm^2. The contents of total soluble solids, glucose and fructose were determined from 4 grape berries for each sample: with refractive device Kruss DR301-95 (% Brix) for total soluble solids (TSS) (Mureşan, 2014; Oltenacu, 2015; Saei, 2011; Tolić, 2015; Yoon, 2005), with refractive device Milwaukee MA873 (%) for glucose and with refractive device Milwaukee MA872 (%) for fructose (Enciu (Bunicelu) et al., 2021). The total 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 (grapes, shoots, bananas). The maturity index, known also like acidometric equilibrium index, was calculated using the formula: TSS/TA. Dehydration and drying of berrie was carried out with the Excalibur 4948CDB dehydrator, 600W used for drying vegetables, fruits, greens, medicinal plants, aromatic plants, vegetable chips and vegetable leaves.

Table 1. The main climatic parameters and bioclimatic indices during the experimentation period

Climatic parameters and bioclimatic indices	Average	Years		Average
	1981-2010	2022	2023	2022-2023
Average annual temperature, ºC	11.55	12.99	14.61	13.80
Average temperature in the growing season (IV-X), ºC	18.07	18.95	21.01	19.98
Average temperature in summer (VI-VIII), ºC	22.50	24.07	27.22	25.64
Average annual minimum temperature, ºC	5.03	5.9	7.1	6.5
Average annual maximum temperature, ºC	17.05	19.8	21.2	20.50
Average maximum temperature in the warmest month, ºC	29.87	32.94	33.9	33.42
Average maximum temperature in summer (VI-VIII), ºC	29.01	32.03	32.86	32.44
Annual total precipitation, mm	608	383.6	435.1	410.35
Total precipitation in the growing season (IV-X), mm	428	281	216.5	298.75
Total precipitation in summer (VI-VIII), mm	198	90.4	93.7	92.05
Huglin index (HI)	2346	3549.6	4054.4	3802
Winkler index (WI)	1726	1898.18	2356.14	2127.16
Cool night index (CNI)	10.45	10.43	14.1	12.26

For this, 2500 g of berrie from the entire harvest obtained from the 9 vines/variety, for each individual sample, were randomly detached from the pedicel. Regarding the determinations made to establish the degree of dehydration was made according to the formula (AOAC 20 013, 1997), and the time was 24 h. In this regard, Moisture content (%) = [(initial weight - final weight)/initial weight]. Establishing the rehydration capacity of dehydrated products as determined by the method proposed by Rozsa, 2019, and using the following formulas. The rehydration ratio Rr represents the ratio between the mass of the rehydrated sample (R) and the mass of the dehydrated sample (D), is calculated according to the relation: RR = the mass of the rehydrated sample/the mass of the dehydrated sample. The water content of the rehydrated sample is as follows: WC = {R-[D-(DxU)]/R} x 100 in which: WC = amount of water of the rehydrated sample, in %; R = mass of the rehydrated sample, in g; D = mass of the dehydrated sample taken as analysis, in g; U = the amount of water contained in one gram of product dehydrated in g. The rehydration capacity represents the percentage of water in the rehydrated material and is calculated according to the relation: Rc = R/[D-U/100-W) in which: Rc = rehydration capacity, in %; W = water content of the fresh product, in %. 400 ml of water were used for rehydration for 100 g raisins. Products that absorb more than 80% of the water lost during dehydration in the rehydration process are considered to be of very good quality, and when the water absorbed is below 50%, the products are considered to be of poor quality (Rozsa, 2019; Călugăr A. et al., 2022). All determinations were performed in triplicate. Statistical analyses were performed using Excel, including: average, standard deviation.

RESULTS AND DISCUSSIONS

Climatic conditions

The analysis of the climatic elements (Table 1) of the 2023 wine year shows high thermal resources, against the background of much too low water resources, at least during the vegetation period (IV-X), with very fluctuating values compared to the 2022 wine year, but also to the average of the last decade (1981-2020) taken into account, as a starting point in assessing the impact of climate change on livelihoods. Practically, since then, the trial of varieties less adapted to the temperate climate has become a source of study and the diversity of vine varieties is an important resource for adapting to climate change (Destrac-Irvine et al., 2020; Antolin et al., 2021). As for the synthetic indicators - Huglin index (HI), Winkler index (WI), Cool night index (CNI), their values were very high, but the largest amplitude was for (HI), which passes from IH4 – warm temperate climate in the arm climate of the type warm IH5 - (IS1, IH5, IF2) (Savu and Stroe, 2005; Savu and Stroe, 2007). For the indicator (CNI), the recorded values are also very high - indicating a climate with cold nights of the IF+1 type (Savu and Stroe, 2007), with the limits between 12^0C and 14^0C at least for the year 2023 and the average of the last 2 years, indicating a possible decrease during the night of some fractions of the quality compounds. Practically the temperatures recorded during the night and the differences from day to night in the last month of the ripening of the grapes leaving their mark, as follows: the TSS, TA values listed in Table 3, are located at the lower limit of its potential according to the data in the genetic passport of the varieties.The evaluation of the quantitative and qualitative performances for the four experimental variants cultivated in/an area different from the one of origin, highlights a behavior that suggests a rather low degree of adaptation, as follows: at the time of harvesting, (4^{th} September 2023), the 'Supernova' variety obtains the highest number of grapes per vine, reflected in a high production per vine and per hectare, respectively 4.73 kg/vine and 17916.66 kg/ha, practically recalling the productivity of the parents - the 'Cardinal' and 'Kişmiş Rozovyi' varieties. Equally, the native variety 'Călina' excels under the conditions of the 2022-2023 wine year with an equally high production, 16,931 kg/ha, being within the productive limits of the variety in the area where it was obtained (Dragăsani, an area with vocation and for apyrene varieties). The 'Sultanina' variety achieves the lowest production, but considering that the specialized literature shows (Stroe, 2012) that this variety has a low production in Romania, the harvest of the wine year in the experimental field reflects an exceptional amount - 1.53 kg/vine and 5,814.39 kg/ha. Regarding the qualitative panel of the harvest presented in Tables 3, 4, a somewhat expected evolution can be observed under the quantity/ quality ratio, highlighted by the accumulation limits of TSS (%) and TA (mg/100 g fw), as follows: the 'Sultanina' variety, which had the lowest harvest, has the highest value of 22.35% (equivalent to 214.7 g/L), which, associated with the acidity, demonstrates a perfect organoleptic balance, illustrated by the gluco-acidometric index value. On the same note, the 'Călina' variety is presented, very productive, but not without balance.

The 'Sublima' and 'Supernova' varieties with 19.76% - 189.6 g/L and 21.17% - 205.5 g/L, against the background of a slightly lower acidity, maintain their quality profile for a more fresh consumption compared to production of raisins. Firmness and pH did not show significant differences between the varieties, the values being very close.

Table 2. Grapes quantitative and qualitative parameters (2022-2023)

Varieties	No. of grapes/vine	Average weight of a grape (g)	Weight of 100 berries (g)	Y/v[1] (kg/vine)	Y/ha (kg)
Calina	23	201.4	148.66	4.47	16.931
Sultanina	10	161.0	130.88	1.53	5814.39
Sublima	10	226.11	246.00	2.20	8344.69
Supernova	31	151.00	202.66	4.73	17916.66

[1]Y/v = Yield/vine (kg)

Table 3. Variation of: TSS (%), glucose (%), fructose (%), TA during the 2022-2023

	TSS [2](%)		**Glucose (%)**		**Fructose (%)**		**TA[3](mg/100 g fw)**	
Samples	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Calina	22.08	±2.39	22.95	±2.37	23.04	±2.33	2.38	±0.16
Sultanina	22.35	±1.10	24.06	±0.99	24.15	±0.89	3.14	±0.22
Sublima	19.76	±2.32	20.58	±2.62	20.73	±2.73	1.86	±0.14
Supernova	21.17	±2.20	21.77	±2.11	22.31	±2.10	1.97	±0.15

[2]TSS% = total soluble solids (Brix); [3]TA% Titrable acidity

Table 4. Variation of: Gluco-acidometric index, ph and fermity during the 2022-2023

	Gluco-acidometric index		**pH**		**Fermity (N/cm^2)**	
Samples	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Calina	9.28	±1.40	3.66	±0.08	0.19	±0.08
Sultanina	7.12	±0.49	3.49	±0.08	0.22	±0.16
Sublima	10.36	±1.60	3.5	±0.05	0.18	±0.08
Supernova	10.72	±1.51	3.41	±0.08	0.20	±0.14

Tabel 5. Variation of degree of dehydration and rehydration capacity for varieties seedlles analyzed (2022-2023)

Formulas	**Parameters**	**Varieties**			
		Călina	**Sultanina**	**Sublima**	**Supernova**
Moisture content (%) = [(initial weight - final weight)/initial weight]	initial weight (g)	2500	2500	2500	2500
	dehydrated weight (g)	732.95	924.87	946.37	946.89
RR = the mass of the rehydrated sample/the mass of the dehydrated sample	water content of the rehydrated sample (g)	1767.05	1575.13	1553.63	1553.11
	water content of the rehydrated sample (%)	70.68	63.01	62.15	62.12
WC = {R-[D-(DxU)]/R} x 100	weight mass fresh for 100 g raisins:	341.09	270.31	264.17	264.02
Rc = R/(D-U/100-W)					
	weight 100 raisins (g)	43.01	48.42	93.23	76.79

Figure 1. Dehydration of berries grapes in Excalibur 4948CDB dehydrator (50^{0}C)

Following the dehydration process carried out with the Excalibur 4948CDB dehydrator, 600W (Figure 1) and using 2500 g of berries from the entire harvest obtained for each individual sample, then listed in Table 5, it shows that the ‘Călina’ and ‘Sultanina’ varieties have the highest dehydration capacity, seen from the perspective of quantity total water lost and a weight mass fresh for 100 g raisins - 341.09 g, respectively 270.31 g. This fact underlines the profile of the two varieties, namely, that of being intended mainly for obtaining raisins and then fulfill the advantage as a table variety. The ‘Sublima’ and ‘Supernova’ varieties, as a result of the obtained data, lose an amount close to the ‘Sultanina’ variety after dehydration, but the amount of weight mass fresh for 100 g raisins is lower.

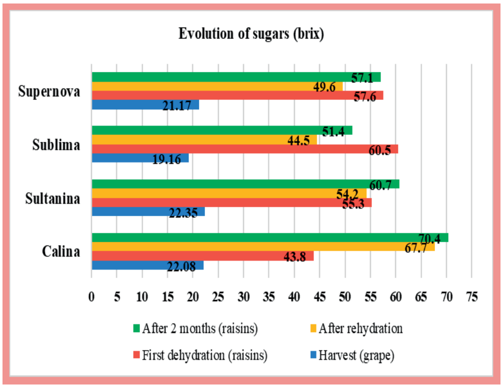

Figure 2. Qualitative limits after rehydration

The results obtained by rehydration (Figure 2). after one month in 400 ml of water show that the trend is opposite to that of dehydration. The varieties ‘Călina’ and ‘Sublima’ assimilate the largest amount of water (Figure 3).

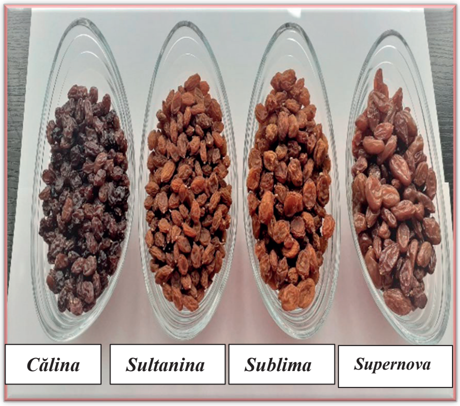

Figure 3. Qualitative raisins for the experimental variants

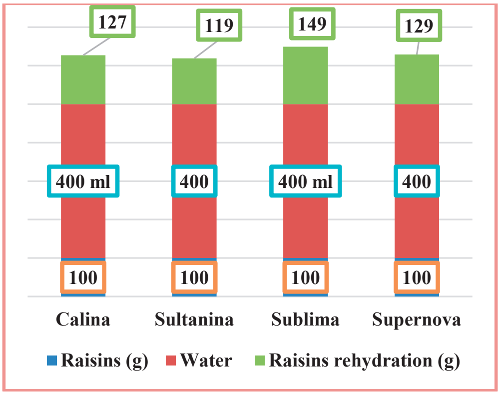

Figure 4. The evolution of sugars in the 4 experimental variants

Regarding the evolution of sugars in the four experimental variants, it can be observed that (Figure 4), the varieties ‘Călina’ and ‘Sultanina’ keep their high values, even two months after obtaining the raisins. This fact highlights once more, that the native variety ‘Călina’ has the profile of a fiery variety for raisins first and then as a table grape. For the ‘Sublima’ variety, the data recorded during this study demonstrate that it is a variety for fresh consumption, quite productive, and on a secondary level it can be used in obtaining raisins or industrialization. The ‘Supernova’ variety surprises in the climatic conditions of Romania, both in terms of productive growth and in terms of quality, having a well-defined mixed profile.

CONCLUSIONS

The behavior of the four varieties in an area quite favorable for grapevine cultivation (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m), reported to the objectives of the study - cultivating them in somewhat cooler areas, once unsuitable for the cultivation of fire varieties, permissive as a solution to climate changes - demonstrates their good adaptability. The 'Supernova' variety surprises in the climatic conditions of Romania, both from a productive and from a qualitative point of view, having a well-defined mixed profile, based on the results obtained.

Following the study, a mixed profile of these varieties can be noted - fresh consumption of the four, less the 'Călina'variety, but also the obtaining of raisins, perhaps for this second quality, at the beginning, only at the artisanal farm level (guest house). Raisins, in this case, can be obtained very easily at home, by using the dryers used in this study.

Although, in the years in which the varieties were obtained, climate change was modestly anticipated and the possibility that they could be cultivated in/an area less friendly for obtaining raisins, the results obtained demonstrate this advantage of them.

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THE ASSESSMENT OF PURITY AND TYPOLOGY OF THE 'PINOT GRIS' CULTIVAR, CULTIVATED IN THE MURFATLAR WINE-GROWING CENTER, THROUGH SYSTEMS BASED ON AMPELOMETRY

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Abstract

A study on ten 'Pinot Gris' cultivar elites was carried on at the Research Station for Viticulture and Enology Murfatlar during the 2021-2022 wine year aimed to authenticate plantation specimens. Ten elites (H1-H10), each with similar growth force, had ten leaves harvested during veraison from the middle part of the shoot. Using a planimeter with 0.1 mm precision, leaf vein length and main angle size were measured. These elites were compared to a control vine from the base plantation. Analysis showed H4, H8, H6, and H9 could be new biotypes for further agrobiological study. H1, H3, and H7 did not differ from the control, suitable for multiplication and maintaining cultivar authenticity. The variation coefficient remained below 30%, ensuring homogeneity and representativeness in the study.

Key words: *ampelometry, typicity, clonal elites, grapevine.*

INTRODUCTION

The distribution of grapevine cultivars in our country stems from the combined action of numerous factors, among which those of particular importance include: the tradition of viticultural regions (Dejeu, 2010; Teodorescu, 1964), the vocation and pedoclimatic potential of different viticultural areas (Oslobeanu et al., 1991), ecological constraints in certain viticultural areas (Bucur et al., 2016; Cichi, 2021; Costea et al., 2008; Irimia et al., 2014; Toti et al., 2017), the varying requirements of grapevine varieties in relation to pedoclimatic factors (Bunea et al., 2017; Dobrei et al., 2016; Rotaru, 2009), as well as technical-organizational restrictions and socio-economic criteria (Olteanu et al., 2002; Cichi, 2022).
Ampelometry represents a method by which grapevine cultivars are identified and described, parameterizing the biometric characteristics of mature leaves through a series of numerical data. The application of this method remains relevant due to the continuous development of information technology and the numerous possibilities for data processing and interpretation, providing valuable insights into the classification of cultivars into different groups, the degree of relatedness between cultivars, as well as their differentiation.

MATERIALS AND METHODS

The research was conducted during the viticultural year 2021-2022 and consisted of studying ten elites of the 'Pinot Gris' cultivar. In order to evaluate the authenticity of the individuals kept in the ampelographic collection of the Research and Development Station for Viticulture and Oenology Murfatlar, the following methodology was applied: Ten vines of the same growth force, referred to as elites (H), were selected, each exhibiting certain characteristics different from those specific to the reference 'Pinot Gris' cultivar (leaf colour, pubescence), labelled as H1 - H10. From each vine, 10 leaves were harvested from the middle third of the shoot, during the veraison phase.
The shoots exhibited the same stage of development and had identical insertion levels. A series of measurements were carried out, focusing on ampelometric characteristics taken from the list of OIV descriptors, namely: The length of the median main vein - N1, length of

the upper lateral main vein - N2, length of the lower lateral main vein - N3, length of the lower secondary vein - N4, angle size between N1 and N2 - α, angle size between N2 and N3 - β, angle size between N3 and N4 - γ, and the following statistical indices were calculated: arithmetic mean, variance, standard deviation, and coefficient of variation.

RESULTS AND DISCUSSIONS

The 'Pinot Gris' cultivar (Figure 1) has a short vegetation period (160-170 days) and moderate vigor, showing good tolerance to frost and drought, and poor resistance to gray mold and powdery mildew. The mature leaf is slightly rounded, medium to small in size, with a thick, wrinkled, dark green lamina, with fine scales. There is a great variability regarding the number of lobes, depth, and shape of the lateral sinuses. Towards the tip of the shoots, there are entire leaves, while towards the base there are leaves with 3-5 lobes. The terminal lobe is short and rounded, and the upper lateral sinuses are closed with an oval lumen or open in a helical shape. In the Murfatlar vineyard, full ripening of the grapes begins in the second decade of September.

Figure 1. Mature leaf of the 'Pinot Gris': cultivar

Ampelometric descriptors target the biometric characteristics of the leaf. In order to determine them, for the 'Pinot Gris' variety, 10 vines of the same vigor, named elites (marked H1- H10), were selected, from which 10 mature leaves were harvested, located in the middle third of the shoot, with the same insertion level on the one-year-old cord, during July-August (veraison phase). The 10 leaves harvested from each vine were copied onto paper, after which, using a planimeter (vernier caliper) with a precision of 0.1 mm, the length of the veins N1, N2, N3, N4 and the size of the main angles α, β, γ were measured and determined (Figure 2).

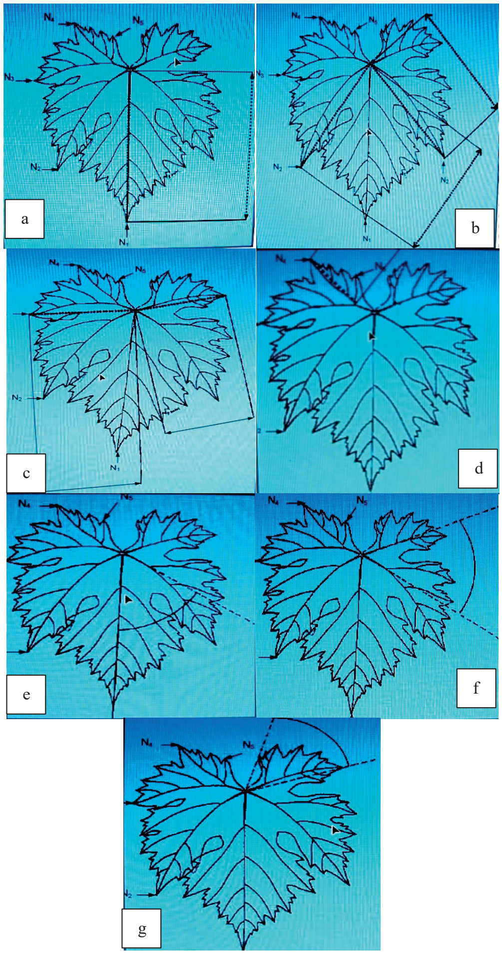

Figure 2. Ampelometric descriptors (after Oșlobeanu et al., 1980): a. Length of vein N1; b. Length of vein N2; c. Length of vein N3; d. Length of vein N4; e. Angle size between N1 and N2, measured at the first bifurcation (α); f. Angle size between N2 and N3, measured at the first bifurcation (β); g. Angle size between N3 and N4, measured at the first bifurcation (γ)

The permanent and evident change in climate factors due to global warming can be determining causes of the variability recorded in the 'Pinot Gris' cultivar in the plantation. The studied cultivar presents a high variability, requiring a continuous selection oriented towards maintaining biological purity as well as towards identifying new valuable biotypes.

Very significant negative differences were recorded in elite H4 for the following traits:

- Length of median main vein N1 (-3.42 cm); Length of upper lateral main vein N2 (-3.56 cm); Length of lower lateral main vein N3 (-2.51 cm); Length of lower secondary vein N4 (-1.46 cm).
- Significant negative differences were recorded in elite H2 for the trait Length of upper lateral main vein N2 (-1.02 cm).
- Very significant positive differences were recorded in elite H8 for the following traits: Length of median main vein N1 (+1.79 cm) and Length of upper lateral main vein N2 (+1.76 cm).

The coefficient of variation had the following values for:

- Length of median main vein N1: moderate value in elite H4 (14.86); low value, ranging from 3.70 to 8.83, in the other variants.
- Length of upper lateral main vein N2: low value, ranging from 3.54 to 9.62 for all elites.
- Length of lower lateral main vein N3: low values (4.06-7.99) for most variants; moderate value for variant H7 (10.65).
- Length of lower secondary vein N4: elites H1, H2, H4, H5, H8, H9, and H10 showed moderate variation with values ranging from 10.65 to 20.00; low variation for the rest of the elites (Table 1, Figure 3).

Table 1 The variability of the ampelometric characteristics (length of veins N1, N2, N3, and N4) in the 'Pinot Gris' variety

Character	**Length of median main vein N1 (cm)**				**Length of upper lateral main vein N2 (cm)**				**Length of lower lateral main vein N3 (cm)**				**Length of lower secondary vein N4 (cm)**			
Estimator Variant	X±Sx	S%	Diff. +/-	Sig.	X±Sx	S%	Diff. +/-	Sig.	X±Sx	S%	Diff.+/-	Sig.	X±Sx	S%	Diff.+/-	Sig.
H1	10.78±0.41	8.56	-0.31	-	9.72±0.36	8.21	0	-	6.80±1.24	7.99	-0.33	-	4.00±0.29	16.20	-0.48	-
H2	9.96±1.39	8.83	-0.51	-	8.70±0.14	3.54	-1.02	0	6.82±0.16	5.23	-0.31	-	4.62±0.22	10.65	+0.14	-
H3	10.74±0.23	4.78	+0.27	-	9.38±0.20	4.85	-0.34	-	6.72±0.09	6.86	-0.41	-	3.98±0.05	2.75	-0.50	-
H4	7.05±0.47	14.86	-3.42	000	6.16±0.20	7.14	-3.56	000	4.62±0.15	7.28	-2.51	000	3.02±0.20	14.55	-1.46	000
H5	11.00±0.17	3.46	+0.59	-	10.48±0.45	9.62	+0.76	-	8.02±0.19	5.32	+0.89	**	5.44±0.28	11.38	+0.96	*
H6	11.14±0.34	6.91	+0.67	-	9.76±0.30	6.90	+0.04	-	6.96±0.22	7.16	-0.17	-	4.20±0.16	8.75	-0.28	-
H7	10.76±0.42	8.73	+0.29	-	9.50±0.18	4.34	-0.22	-	6.66±0.31	10.65	-0.47	-	4.04±0.13	7.54	-0.44	-
H8	12.26±0.32	5.81	+1.79	***	11.48±0.37	7.11	+1.76	***	7.90±0.27	7.65	+0.77	**	4.48±0.34	16.96	0	-
H9	11.24±0.39	5.32	+0.77	*	10.38±0.39	8.35	+0.66	-	7.80±0.21	6.08	+0.67	*	5.12±0.27	11.83	+0.64	-
H10	9.86±0.16	3.70	-0.61	-	8.92±0.25	6.21	-0.80	-	6.66±0.12	4.06	-0.47	-	4.46±0.41	20.71	-0.02	-
CONTROL	**10.47± 0.35**				**9.72± 0.18**				**7.13± 0.16**				**4.48±0.21**			
DL	DL 5% = 0.96 DL 1% = 1.30 DL 0.1% = 1.72				DL 5% = 0.85 DL 1% = 1.14 DL 0.1% = 1.51				DL 5% = 0.62 DL 1% = 0.84 DL 0.1% = 1.11				DL 5% = 0.73 DL 1% = 0.98 DL 0.1% = 1.29			

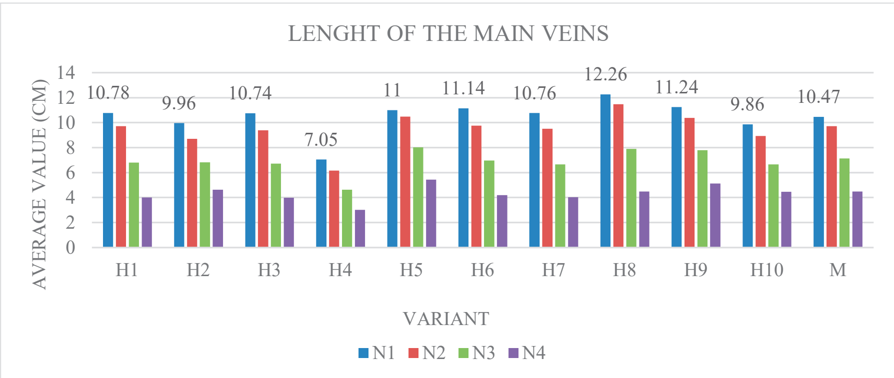

Figure 3. Graphic representation of the variant for the characteristic Length of veins N1, N2, N3, N4 compared to the control

Following the analysis of the significance of differences compared to the control regarding the ampelometric characteristics (angle size), the following observations were made:

- Very significant negative differences were recorded in elite H4 for the following traits: Angle size α between N1 and N2 (-18°13´); Angle size γ between N3 and N4 (-14°9´).
- Distinctly significant negative differences were recorded for the trait: Angle size α between N1 and N2 (-5°7´) in elite H9; Angle size β between N2 and N3 (-11°18´) in elite H4.
- Significant negative differences were recorded for the trait: Angle size α between N1 and N2 in H10 (-4°8´); Angle size γ between N3 and N4 in H6 (-6°7´).

The coefficient of variation had the following values for:

- Trait Angle size α between N1 and N2: Low variation in elites: H1 (7.86); H3 (8.15); H8 (5.48); H9 (6.29) and H10 (9.46); Moderate variation in elites H2 (10.50), H5 (11.22), H6 (12.25), and H7 (10.94); High variation in elite H4 (20.33).
- Trait Angle size β between N2 and N3: Low value in elites H2, H6, H8, H9, and H10 ranging from 5.08 to 9.20; Moderate value in elites H3, H5, H7 (10.76-13.20); High value in elite H4 (20.95).
- Trait Angle size γ between N3 and N4: Low variation in elites H1, H5, H7, H8, H9, with values ranging from 5.42 to 9.09; Moderate variation in elites H2, H3, H4, H6, and H10, with values ranging from 11.35 to 14.49 (Table 2, Figure 4).

Table 2. Variability of ampelometric characteristics (angle size α, β, γ) in the 'Pinot Gris' cultivar

Character	**Angle size between N1 and N2 (α)**				**Angle size between N2 and N3 (β)**				**Angle size between N3 and N4 (γ)**			
Estimator Variant	X±Sx	S%	Diff.+/-	Sig.	X±Sx	S%	Diff. +/-	Sig.	X±Sx	S%	Diff.+/-	Sig.
H1	59.4±2.09	7.86	+4.7	*	53.00±2.55	10.76	+2.96	-	53.40±1.89	7.20	+2.1	-
H2	57.4±2.69	10.50	+2.7	-	55.00±2.10	8.54	+4.96	-	55.20±3.18	12.90	+39	-
H3	55.80±2.03	8.15	+1.1	-	54.20±3.20	13.20	+4.16	-	52.20±3.38	14.49	+0.9	-
H4	36.57±3.33	20.33	-18.13	000	38.86±3.64	20.95	-11.18	00	36.31±1.97	12.16	-14.9	000
H5	56.00±2.81	11.22	+1.3	-	48.20±2.52	11.68	-1.40	-	49.20±1.32	6.00	-1.2	-
H6	53.40±2.93	12.25	-1.3	-	46.60±1.96	9.43	-3.44	-	44.60±2.77	13.88	-6.7	0
H7	59.00±2.89	10.94	+4.3	*	55.20±3.08	12.51	+5.16	-	59.40±2.20	8.29	+8.1	-
H8	53.80±1.32	5.48	-0.9	-	47.00±1.70	8.12	-3.04	-	55.00±2.24	9.09	+3.7	-
H9	49.00±1.38	6.29	-5.7	00	44.00±1.00	5.08	-6.04	-	47.80±1.16	5.42	-3.5	-
H10	49.80±2.11	9.46	-4.8	0	51.00±2.10	9.20	+0.96	-	47.20±2.40	11.35	-4.1	-
CONTROL	**54.70**				**50.04**				**51.30**			
DL	DL 5% = 4.04 DL 1% = 5.41 DL 0.1% = 7.14				DL 5% = 7.08 DL 1% = 9.49 DL 0.1% = 12.53				DL 5% =6.73 DL 1% =9.03 DL 0.1% =11.91			

Figure 4. Graphic representation of the variant for the angle size characteristic compared to the control

Comparative analysis of ampelometric descriptors (length of veins N1, N2, N3, N4 and size of angles α, β, γ) represented an important stage in evaluating the authenticity of the studied variety.

Positive differences in leaf size and angle size, compared to the control, recorded for the elites H4, H6, H8, and H9, reveal that for these, the growth is greater, resulting in an average shoot length of over 150 cm, with good cord maturity (90%).

The leaf blade performs functions of particular importance for the plant, therefore in the case of these four elites, the recorded differences had positive effects on grape quality, as they were well-developed, uniform, with an average weight of 92 g, sugar content was 204 g/l, and acidity was 4.8 g/l H_2SO_4. Yields in this case were both qualitative and quantitative, averaging 3 kg/bunch.

Correlating these traits with the analysis of the variability of ampelometric characters, in the case of elites H4, H6, H8, and H9, it emerges that these can constitute new biotypes and can be studied further for the evaluation of agrobiological traits.

Considering the comparative study for elites H1, H3, and H7 as a whole, the recorded differences are insignificant compared to the control, the growths were within normal limits, and grape quality was superior, therefore these elites constitute the basis for multiplication and maintenance of the authenticity of the 'Pinot Gris' variety.

The coefficient of variation did not exceed the threshold of 30%, all samples fell within the limits of homogeneity, being representative of the individuals included in the study.

CONCLUSIONS

The biometric data, upon which the corresponding grades were given, reveal significant differences among the studied elites regarding the size and shape of the leaf, given by the length of the main veins N1, N2, N3, N4, the length L and the width l of the lamina, the size of the main angles α, β, γ between the main veins, justifying the choice of leaves as the main morphological and ampelographic characteristic for the identification and description of grapevine varieties.

The proposed descriptors support the differentiation of very similar varieties in terms of other characteristics or the identification of new biotypes.

Analysing and correlating the statistical data for the 'Pinot Gris' variety, it can be concluded that out of the 10 studied elites, only four could constitute new biotypes, namely, H4, H8, H6, and H9, which present clear differences compared to the control variety.

Differences in the size and shape of the leaves as well as the size of the angles reveal that for these elites, the growth is greater, resulting in an average shoot length of over 150 cm, with good cord maturity (90%). Implicitly, the quality of the grapes is superior, and the yields are both qualitative and quantitative, averaging 9-13 kg/ha.

The studied elites demonstrate good adaptability to current climate changes, making them suitable for cultivation in the Dobrogea region.

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VEGETABLE GROWING

EX·TERRA·AVRVM

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A BRIEF DESCRIPTION OF CULTIVATED CHILI PEPPERS

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Abstract

Chili peppers is an important vegetable and spice crop grown all over the world for fresh, dried, and processing products. Capsicum spp. is a member of the Solanaceae family and native to the temperate, subtropical and tropical regions of the Americas. This research paper provides a comprehensive overview of the distinctive descriptors of the Capsicum genus, showcasing images sourced from the germplasm collection at the Vegetable Research Development Station (VRDS) Buzau, Romania. The paper focuses on morphological features as the key basis for differentiation among the domesticated Capsicum species: C. annuum L., C. chinense Jacq., C. frutescens L., C. baccatum L. şi C. pubescens Ruiz & Pav. Various descriptors, such as flower and fruit shapes, corolla color, corolla sport color, the presence or absence of calyx annular constriction, and seed colour, are employed to provide a nuanced understanding of the unique characteristics of each species. By elucidating these morphological features, the paper aims to empower readers to easily distinguish between different Capsicum varieties.

Key words*: biodiversity, Capsicum spp., morphological features, Solanaceae.*

INTRODUCTION

The genus *Capsicum* was one of the first genera to be domesticated, around 6000 B.C by Native Americans (Perry et al., 2007), and is now produced at over 35 million tons per year worldwide (FAO, 2021).

Fruits of the genus have good health properties such as fat breakdown and stress relief (Shiragaki et al., 2020). Besides being used fresh and in a broad variety of dishes in international cuisine, hot peppers are a raw material of many industries such as food industry (powder, sauces, pastas), pharmaceutical (creams, patches, sprays, ointments), chemical (protective coatings for electrical wires and as an additive in ship paints), and the military (projectiles, self-defence sprays) (Muñoz-Ramírez et al., 2020).

The genus comprises 43 described species (Barboza et al., 2020), with broad diversity in the colour, shape and size of the fruit, in the sensory attributes such as aroma, taste, and hotness, and levels of pungency (Cardoso et al., 2018). Among these species, five are considered domesticated: *C. annuum* L., *C. baccatum* L. (var. *pendulum), C. chinense* Jacq., *C. frutescens* L., *C. pubescens* Ruiz & Pav. (DeWitt and Bosland, 2009).

The origin of the *Capsicum* genus is presupposed to be along the Andes of western to north-western South America (Carrizo García et al., 2016). According to Pickersgill et al. (1971), three of the domesticated species, *C. annuum*, *C. frutescens*, and *C. chinense* form a closely related group that evolved in the lowlands of the tropics of Latin America and the Caribbean, with *C. annuum* predominating in Mexico, *C. chinense* in the Amazon basin and *C. frutescens* in the Caribbean.

Chili peppers crossed the Atlantic from Mexico to Europe, Christopher Columbus is supposed to have brought to Spain the first samples of chili pepper. Unlike the tomato, often perceived as poisonous, the chili pepper started being cultivated in many areas of Southern Europe soon after its introduction (Katz, 2009).

In Romania, peppers arrived in the XVIII century, and the first varieties of cultivated peppers were mentioned by Alessiu (1894). The common species of pepper predominantly cultivated in Romania is *Capsicum annuum* L. Sweet peppers are the major cultivars released through production and commercialization in Romania, followed by long peppers and hot peppers. Currently, the food industry is actively seeking healthy products, redirecting consumer perception, attitude, and awareness toward a diet rich in natural and high-quality products (Amicarelli et al., 2021). This shift in consumer interest is why the cultivation of hot peppers has significantly increased among Romanian smallholder and hobby farmers in recent years.

MATERIALS AND METHODS

In this research paper, our primary objective is to present a comprehensive overview of the five domesticated *Capsicum* species housed at the Vegetable Research Development Station (VRDS) Buzau. Our aim is to facilitate the differentiation of these species based on distinct morphological features. Additionally, we seek to provide a user-friendly guide for enthusiasts of *Capsicum* varieties, enabling them to easily distinguish and appreciate the unique characteristics of each species.

The VRDS Buzau holds a pivotal role in the preservation and breeding of peppers in Romania, particularly contributing to the conservation of genetic resources. The extensive germplasm collection at VRDS Buzau encompasses a diverse array of 500 genotypes (refer to Figure 1), further emphasizing the station's significance in maintaining and studying the rich biodiversity of *Capsicum* species.

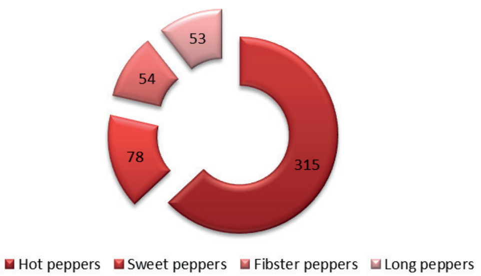

Figure 1. Structure of the *Capsicum* germplasm collection from VRDS Buzau

RESULTS AND DISCUSSIONS

The cross-pollination rate among *Capsicum* spp. is highly variable, ranging from 2 to 90% (Justino et al., 2018). The five cultivated species of *Capsicum* are self-compatible and diploid, with the number of chromosomes in the species being 2n = 24 (Pozzobon et al., 2006). Some wild species have 26 chromosomes (Pickersgill, 1991; Tong and Bosland, 2003). This can be morphologically differentiated based on qualitative characters, such as floral characteristics (Figure 2).

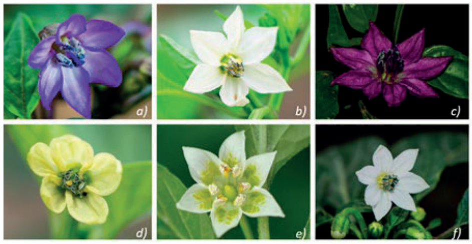

Figure 2. Distinctive inflorescences of the genus *Capsicum:* a), b), c) *C. annuum*, d) *C. chinense*, e) *C. baccatum*, f) *C. frutescens*

C. chinense species presents two or three flowers per leaf node of a whitish-green color, with a constriction at the base of the calyx (Figure 3) at the junction with the pedicel. *C. frutescens* species presents erect white-green flowers without calyx constriction, while *C. annuum* has creamy white or blue flowers (Hernández-Pérez et al., 2020; Paredes Andrade et al., 2020).

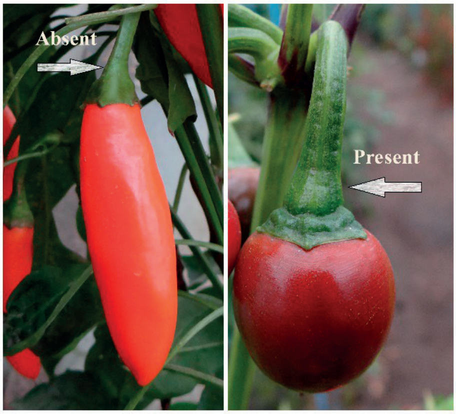

Figure 3. Calyx annular constriction

The other two species are relatively easy to distinguish: *C. pubescens* has black-rough seeds (Figure 4) and either uniformly purple or purple flowers with a white base. *C. baccatum* has yellow or white-yellow flowers with greenish spots towards the basal part of the petals (Paredes Andrade et al., 2020).

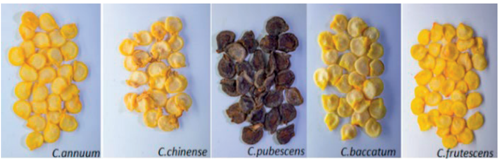

Figure 4. Seeds of *Capsicum* species

Furthermore, the differentiation of the *Capsicum* cultivated species is based on morphological descriptors that define the shape of flowers and fruits (Sudré et al., 2010). For accurate characterization, other descriptors are considered essential, such as those indicated by IPGRI. They can also be identified by the different flavors and pungency of the fruits (Comparini et al., 2021).

However, the classification and recognition of chilies, like that of any multifarious group of cultivars, is confusing. In this paper, we provide an overview of the five domesticated *Capsicum* species to differentiate them based on morphological features.

Capsicum annuum L.

C. annuum L. is the most popular and extensively cultivated pepper worldwide (de Carvalho et al., 2006; Hernández-Pérez et al., 2020). This species is characterized by both non-pungent and pungent accessions (Figure 5) with a sub-shrub or herb growth habit. The fruits exhibit variations in size, shape, and colours at maturity (Tripodi and Kumar, 2019). Some cultivars of *C. annuum* are well-accepted as ornamental plants due to the bright and colourful fruits and leaves (Costa et al., 2019).

C. annuum typically has erect plants that grow 1-1.5 meters tall. The leaves are simple and similar in size and shape. The leaf blade is ovate to elliptic, with a margin that ends in an acuminate or long-acuminate apex. The inflorescences are axillary, with one flower per axil, rarely more. The corolla is usually entirely white, occasionally entirely purple or pale yellow, with white or cream filaments, sometimes purple, and pale blue to purplish anthers.

The berries are highly variable in size, shape (elongated, round, triangular, campanulate, or blocky), and colour (white, yellow, green, orange, purple, and deep purple when immature; lemon-yellow, pale yellow-orange, pale orange, orange, light red, red, dark red, purple, and purple-black at maturity). The seeds are pale yellow, with more than 50 seeds per fruit (Barboza et al., 2022).

a) b) c) d)

Figure 5. *C. annuum* varieties from VRDS Buzau, Romania:
Hot peppers: a) Roial; b) Decebal;
Sweet peppers: c) Regal; d) Cantemir

Capsicum baccatum L. var. _pendulum_

Capsicum baccatum L. var. *pendulum* is primarily cultivated in South America and is a plant well-adapted to diverse ecological conditions, ranging from 150 to 3,400 meters in elevation (Djian-Caporalino et al., 2007; Cardoso et al., 2018). *C. baccatum* var. *pendulum* is characterized by erect plants that can exceed a height of 2 meters. The leaf pairs are unequal in size but similar in shape, with membranous leaves that are slightly discolorous. The leaf blades are ovate, with entire margins and an acute apex. The inflorescences are axillary, with 2-3 flowers per axil, and occasionally solitary flowers. The flowers are campanulate, with a white corolla featuring greenish-yellow spots. The filaments are white, inserted on the corolla, and the ellipsoid anthers are white or pale yellow, occasionally greyish, and not connivant at anthesis.

The berry (Figure 6) is globose or subglobose, less frequently ellipsoid with a truncate or flattened apex. It is green when immature, turning to greenish-black and then bright red at maturity. The berry is deciduous, pungent, with a thick, opaque pericarp containing giant cells. Seeds are ovoid, subglobose, or C-shaped, pale yellow to yellow, with 12-15 seeds per fruit (Barboza et al., 2022).

a) b) c) d)

Figure 6. *C. baccatum* var. *pendulum* varieties from VRDS germplasm collection: a) L36; b) L135A; c) L135B; d) L135C

Capsicum *chinense* Jacq.

The fruits of the cultivar Pepper X, belonging to *Capsicum chinense* Jacq., have officially earned the title of the world's hottest chili according to the Guinness Book of World Records (2023). *Capsicum chinense* thrives in wet tropical and subtropical forests, typically found at elevations ranging from 100 to 800 meters (Sarwa et al., 2012).

C. chinense is characterized by an erect plant, standing 0.5-2.5 meters tall, with few to many branches originating near the base. The leaf pairs are unequal in size but share a similar shape, with membranous, either concolorous or slightly discolorous leaves - dark green above and pale green below. The leaf blades are ovate to elliptic, featuring entire margins and a short-acuminate, acuminate, or acute apex.

Inflorescences are axillary, typically hosting 2-4 flowers per axil, occasionally with solitary flowers. The corolla is 5 (6)-merous, dull white or greenish-white, occasionally adorned with purple spots both outside and within. The corolla is stellate with an interpetalar membrane, and the filaments come in white, cream, or purple, inserted on the corolla. Anthers are usually blue or bluish-grey, with rare instances of yellow or greenish-white, broadly ellipsoid or ellipsoid, and connivent or not connivent at anthesis.

The berry, measuring less than 10 mm in diameter, is subglobose and orange to red in wild populations. Highly variable in size, shape, and colour, it can be subglobose, triangular, long-triangular, campanulate, or even blocky. The apex may be pointed, blunt, or long-acuminate and upcurved, while the base is obtuse or truncate. When immature, the colour ranges from green, yellow, brown, to purple, transitioning to pale yellow, yellow, dark brown, orange, red, or vermilion-scarlet at maturity (Figure 7). The fruits may be deciduous or persistent and are known for their intense pungency, though occasional non-pungent varieties exist. Seeds are C-shaped or subglobose, pale yellow, or nearly white, numbering from 14 to 35 per fruit. Notably, fruits of *C. chinense* exhibit a robust annular constriction at the junction with the pedicel (Barboza et al., 2022).

a) b) c) d)

Figure 7. *C chinense* varieties from VRDS Buzau germplasm collection: a) L110; b) L137; c) L76; d) L127

***Capsicum frutescens* L.**

Capsicum frutescens L. thrives in low semi-deciduous forests, disturbed areas, and agricultural clearings, with distribution spanning South America and other tropical and subtropical regions such as Asia, Africa, and the Pacific Islands. It is commonly cultivated near homesites and in the chakras of local communities, spanning elevations from 10 to 2,000 meters. The highly pungent fruits are known for their specific aroma and are consumed either fresh or as a spice (Yamamoto and Nawata, 2005).

C. frutescens is characterized by its small, elongate, narrowly triangular, and upright spicy fruits, featuring a thin fruit wall that typically turns red when ripe. Notably, there is no calyx annular constriction between the calyx and the pedicel (Carvalho et al., 2014). The plant's height ranges between 0.3 to 1.5 meters, branching from near the base. The leaf pairs are more or less similar in size and shape, with membranous, concolorous, or slightly discolorous leaves that are glabrescent or glabrous on both surfaces. The blades of the leaves are ovate or narrowly elliptic, with entire margins and an acuminate to long-acuminate apex.

Inflorescences are axillary, typically hosting 2-5 flowers per axil, with solitary flowers being a rare occurrence. The corolla is usually dull white or greenish-white both outside and within, stellate with an interpetalar membrane. Filaments come in cream or purple, and anthers are ellipsoid, bluish-grey, purplish, or, rarely, dark green or yellow, connivent at anthesis. The berry is typically green and yellowish-green when immature, transitioning to yellow, orange-red (Figure 8) as it matures. Seeds are C-shaped to subglobose, pale yellow, numbering from 10 to 52 per fruit (Barboza et al., 2022).

a) b) c) d)

Figure 8. *C. frutescens* L. varieties from VRDS Buzau germplasm collection: a) L33; b) L23; c) L261; d) L91

Capsicum pubescens **Ruiz & Pav**

C. pubescens Ruiz & Pav is morphologically and genetically distinctive compared to the other four cultivated species of the Capsicum genus (Russo, 2012). *Capsicum pubescens* is more tolerant to low temperatures and is frequently found in the Andean mid-elevations to highlands, ranging from 8000 to 3500 meters and rarely below 500 meters elevation. The phytotoxic activity of *C. pubescens* extracts on weeds of the genera *Amaranthus, Bidens*, and *Ipomoea* is reported. Additionally, its fruits can be considered as nutraceuticals due to their content of phenolic compounds (García-Ruiz et al., 2013).

The plant habit is erect, reaching a height of 1 to 4 meters. The leaf pairs are markedly unequal in size, with similar or dissimilar shapes. The leaves are membranous, concolorous or discolorous, glabrescent to densely pubescent on both surfaces and margins, with trichomes similar to those on stems. The leaf blades are ovate or, more rarely, elliptic, with an asymmetric and attenuate or cuneate base, entire margins, and an acuminate apex.

Inflorescences are axillary, typically hosting 1-2 flowers per axil, rarely up to four flowers. The corolla is dark purple or violet with a white center outside and within (sometimes with a weak yellowish-green center within). It is rotate to stellate, with a thin interpetalar membrane, pubescent adaxially with short glandular trichomes, and glabrous abaxially. Filaments are purple or lilac, inserted on the corolla, and anthers are ellipsoid or ovoid, purple with a wide cream connective, not connivent at anthesis.

The berry is 20-40 mm long, round, blocky, or elongate-curved, with the base obtuse, lobate or truncate, sometimes narrowed forming a neck-like structure, and the apex blunt or sunken, rarely pointed. It is green when immature and brightly colored at maturity, ranging from red to light yellow or blackish (Figure 9). The berry is persistent, very pungent, with a thick pericarp. Seeds are brownish-black to black, C-shaped, subglobose, or irregular, numbering from 15 to 45 per fruit (Barboza et al., 2022).

a) b) c) d)

Figure 9. *C. pubescens* varieties: a) Manzano; b) Brown Rocoto; c) Amarillo; d) Rocoto (Source: Dhamodharan et al., 2022)

CONCLUSIONS

The evaluation of genetic diversity within a *Capsicum* spp. germplasm collection traditionally relies on both qualitative and quantitative morphological descriptors.

The distinction among cultivated *Capsicum* species is achieved through morphological characteristics such as flower and fruit shape, corolla colour, corolla sport colour, the presence or absence of calyx annular constriction, and seed colour.

These morphological descriptors serve as valuable tools for characterizing and differentiating various *Capsicum* species, providing insights into their genetic variations and contributing to the overall understanding of the germplasm collection.

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RESEARCH ON THE EFFECT OF SALINITY ON TOMATO (*Lycopersicon esculentum* Mill.) DURING THE SEED GERMINATION STAGE AND ON THE VEGETATION PERIOD: REVIEW

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Abstract

The tomato is a major annual crop that is grown all over the world for the nourishment of consumers. Since it is predicted that by 2050, over 50% of arable land will be saline, researchers have recently concentrated on understanding how tomato plants respond to different saline environments. The tomato is regarded as "moderately tolerant" of salinity because of its capacity to maintain ionic and water balance in the root zone at moderate salinity levels and because it is more vulnerable to salt stress than its wild equivalents. Some papers include information on how different cultivars behave under different salinity concentrations, analyse various parameters, and discuss the mechanisms regarding tomato salt tolerance. When the salt concentration increased, tomato seed germination was reduced, the time required for full germination was prolonged, plant growth and productivity were limited, and sometimes it led to plant death. Therefore, this review provides a synthesised understanding of the latest scientific findings about the impact of salinity on tomato fruit morphology (germination and seedling growth), physiological (transpiration), biochemical characteristics, as well as yield and fruit quality indicators.

***Key words**: genotypes, tomato seeds, salinity stress, germination.*

INTRODUCTION

Plants are always exposed to their surroundings due to their sessile nature. For plants to carry out vital processes like photosynthesis and to develop vegetatively and reproductively, they must adjust to their ever-changing surroundings (Mundaya Narayanan et al., 2023). A global issue, salt in the soil endangers crop growth and productivity while impeding the long-term viability of contemporary agriculture. Soil salinity is mostly caused by high salinity groundwater levels rising and by inadequate irrigation and drainage systems (Rengasamy, 2006). Currently, 632 million hectares of arable land, or one-fifth of all cultivable soil on Earth, are categorized as being damaged by salt.

In the context of agricultural production, saline and alkaline soils are commonly referred to as salt or salty soils. They cover roughly 250,000 hectares in our country, with the largest areas being found in the Danube Meadow, the Plain in the west of the country, the Romanian Plain, the Moldavian Plateau, the area around the Black Sea coast, and the meadows of several inland rivers (Olt, Jiu, Siret, etc.) in the Jijia-Bahlui Depression.

In general, saline soils with solution osmotic pressures greater than 10-12 atmospheres inhibit plant growth. The detrimental impact of salts on plants is reliant with the specific composition of the salts as well as their concentration in the solution. (Läuchli & Grattan, 2007; Covașă et al., 2023). The salinity of arable land is becoming a greater problem in many irrigated, semi-arid, and arid regions of the world where rainfall is insufficient to wash away salts from the root zone. This contributes significantly to decreased crop yield (Francois & Maas, 1999; Van Zelm et al., 2020). However, since salt destruction depends on species, variety, development stage, environmental factors, and the nature of the salts, it is difficult to define saline soils precisely. According to Ponnamperuma (1984), saline soils are those that have enough salt in the root zone to impede crop plant growth. In the Dictionary of

Environment and Conservation, saline soil is defined as soil that contains enough soluble salts (sulphates and chlorides of sodium and calcium) to reduce its fertility. According to Jamil et al. (2011), a saline soil is commonly characterized as one with an exchangeable salt content of 15% and an electrical conductivity (EC) of the saturation extract (ECe) at the root zone above 4 dS m^{-1} (about 40 mM NaCl) at 25°C. The presence of water-soluble salts such as sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), and sulphate ($SO4^{2-}$) constitutes salinity in soil. Some ions, such as K^{+} and $SO4^{2-}$, are thought to be nutrients for plants, although Na^{+} and Cl^{-} are not. As a result, Na^{+} and Cl^{-} are frequently the focus of soil salinity (Stavi et al., 2021). Läuchli and Lüttge (2002) defined salinity as the "concentration of dissolved mineral salts presents in soils (soil solutions) and waters". To sum up, soil salinity is a measurement of the concentration of all soluble salts in soil water and is commonly given in decisiemens per metre (1 dS m) as the electrical conductivity (EC) of the saturation extract (ECe) (Hasanuzzaman et al., 2013).

A large amount of salt in the soil inhibits the plant's capacity to absorb water, resulting in osmotic stress and ion toxicity due to the excessive accumulation of Cl^{-} and Na^{+} (Leyva et al., 2011; Parihar et al., 2015; Yang & Guo, 2018; Zulfiqar et al., 2022). Salinity has an indirect effect on plant development through the degradation of soil physical conditions (Driessen et al., 2001), but it also directly affects plant growth by influencing plant water uptake, and nutrient availability (Litalien & Zeeb, 2020). At the molecular and biochemical levels, plants have a few defence mechanisms against salinity stress (Shiozaki et al., 2005; Hauser and Horie, 2010).

Munns (1993), specifies that the reaction of plants to salinity takes place in three phases: the zero phase is characterized by the adaptation of plants to a short-term osmotic shock to the stress triggered by salts; in the second phase, there is a reduction in growth due to the osmotic stress caused by the salts in the external solution at the root level; and in the third phase, there is a change in the intensity of transpiration, which occurs depending on the genotype and the ionic toxicity at the foliar level. Thus, the increased concentration of salts reduces the osmotic potential of water around the root system, ultimately making it difficult for plants to absorb water (osmotic effect) and induces symptoms of toxicity (ionic effect), resulting from the metabolic balance and premature senescence of the leaves. During the three phases, a series of molecular and physiological changes can be observed, respectively: a decrease in stomatal conductivity and root water conductance, readjustment of the water balance by synthesizing some osmoactive compounds, readjustment of growth rate, nutritional disorders, as well as a change in the concentration of photoassimilating pigments (Munns & Tester, 2008).

The tomato, or *Lycopersicon esculentum* Mill., is a self-pollinating vegetable crop that is grown all over the world and is considered to be one of the most valuable crops in the vegetable family Solanaceae.

Tomatoes have been described as "moderately tolerant" of salinity because they are capable of preserving ionic and water balance in the root zone at moderate salinity levels. In addition to being a model plant for studies on stress tolerance, genetics, and fruit development, its fruits are extensively consumed in the fresh market (Rothan et al., 2019). It is essential for providing the human diet with an impressive amount of vitamins A and C. It also has a high lycopene content, which may help mitigate the negative effects of free radicals, which are believed to be linked to several cancers and age-related disorders.

The success of seedling establishment and subsequent development is dependent on seed germination, which is an essential and crucial stage in the plant's life cycle. It is a multi-stage, complex developmental process that is influenced by internal as well as external factors. Seed size, age, chromatin-associated elements, proteins, plant hormones (auxin), related genes (maturating and hormonal genes), non-enzymatic processes, and structural components (endosperm and seed coat) are examples of internal variables. In addition, the germination of seeds is influenced by external factors such as moisture, light, salinity, temperature, acidity, and nutrients (Finch-Savage & Leubner-Metzger, 2006; An & Lin, 2011).

Even though seed germination under salt stress is crucial, little is known about the mechanism(s) behind seeds' ability to tolerate salt. Salt stress in vegetative plants results in decreased rates of root and leaf elongation as well as decreased cell turgor, indicating that saline in the environment predominantly affects water intake (Fricke et al., 2006). Both the embryo and the endosperm interact in two ways throughout the germination process: the embryo controls the endosperm's deterioration, while the endosperm serves as an environmental sensor to control the embryo's growth (Yan et al., 2014). Furthermore, germination is regulated by a variety of interrelated hormonal and physical variables (Chahtane et al., 2017).

Tomato seed embryos are surrounded by endosperm and testa, acting as mechanical barriers for radicle protrusion. Endosperm weakening and testa degradation are necessary for germination. Germination of tomato seeds takes place only at temperatures above the threshold of 9-10ºC, but below 35, with the optimum occurring at temperatures between 20 and 25ºC. The lower the germination temperature, the longer the duration of this process, increasing the risk of seed disease (Munteanu, 2003; Inculet & Stoleru, 2021).

Numerous scientific studies have observed that the highest percentage of seed germination occurs in conditions with distilled water and that the rate decreases with increasing media salinity (Singh et al., 2012; Ratnakar & Rai, 2013; Rofekuggaman et al., 2020; Adilu & Gebre, 2021). Salt stress affects tomato seed germination by altering key enzyme activities and gibberellin levels, thereby delaying and reducing the germination rate (Singh et al., 2012; Tanveer et al., 2020). Tomato seeds were severely injured by salinity stress due to decreased germination and an elevated relative injury rate. Under salinity circumstances, the seedling vigor index and height also had a considerable decrease (Choudhury et al., 2023). In addition, some authors suggested that moderate salinity enhances fruit quality by influencing the pH value and levels of soluble solids, such as sugars and acids; these are important variables in determining the quality of fruit offered in markets, and salt stress typically enhances fruit quality by raising the content of those components (Coban et al., 2020; Ladewig et al., 2021).

In the light of this circumstance, a review was carried out to determine the impact of sodium chloride stress on seed germination, the subsequent development of seedlings, but also on the entire vegetation period of tomato plants.

MATERIALS AND METHODS

This summary focuses on an in-depth investigation of the resources that are accessible through the international literature. The data for this analysis was collected from accessible resources on the internet, including Google Academic, Free Full PDF, Research Gate, Science Direct, MPDI, Frontiers, Web of Science, PubMed, and the International Society for Horticultural Science. To identify the content of our work, we searched for phrases and terms like "growth", "vigour index", "salt tress", "seed germination", and "screening of salinity effects".

In this study, only original scientific publications establishing the effects of individual salinity on morphology, physiology, yield, and fruit quality that were published in scientific journals with peer review during the last few years were included. When searching for scientific publications using the keyword "salinity effects on tomato", 106 000 results were retrieved on Google Academic; however, only 335 unique works were found in PubMed databases. After carefully reviewing the titles and abstracts, papers that were determined to be unrelated to the search topic were eliminated. The full texts of the remaining papers were then downloaded and evaluated in order to determine whether they fulfilled the established standards. Based on these criteria, 98 research papers and experiments had an influence on the morphology, physiology, and phytochemicals of tomatoes under salt stress.

RESULTS AND DISCUSSIONS

Currently, research has demonstrated that varying salinities in soil or irrigation water can alter plant morphology, physiology, and biochemistry, with specific implications for fruit quality and output. Excessive levels of salt may also cause imbalances in the absorption and utilisation of vital nutrients like magnesium, calcium, and potassium, which can affect the nutritional status of plants and their general health.

The effect of salinity on tomato morphology

Salinity has a profound effect on each aspect of a tomato plant's being, altering even its physical traits. Generally speaking, a plant's morphology provides information about its metabolic function by reflecting the environmental factors that affect it (Roșca et al., 2023). The cultivation of species in a saline environment depends on the capacity of plants to adapt to salty water during germination and the early phases of seedling development.

During the vegetative stage, the optimal EC range for tomatoes produced in soilless or hydroponic systems is usually 1.5 to 3.0 milli Siemens per centimetre (mS/cm). When the plants reach the fruiting and blooming stages, they may tolerate a small increase in EC, up to 2.0-4.0 mS/cm. These levels prevent excessive salt accumulation while offering a suitable balance of nutrient availability and absorption. The most vulnerable stage of a plant's life cycle is the seedling stage, and germination controls when and where seedling growth starts. Regarding the relative susceptibility of germination and seedling growth to salt stress, there are contradictory observations in the literature. Singh showed in a 2012 study carried out in New Delhi that tomato seed germination decreased at a relatively low salinity (1% NaCl). Germination percentages in parents dropped sharply from 77.60% (control) to 29.60% (at 3% NaCl) and in F1s from 75.82% (control) to 33.16% (at 3% NaCl) at higher salinity (3% NaCl). Shanika & Seran (2020) found that seed treated with NaCl above 40 mM had a germination percentage of less than 50%, with the lowest figure (18.3%) occurring at 80 mM salinity. Following a 12-day period of seeding, salt stress had a substantial ($P<0.001$) impact on the shoot and root lengths, dry and fresh weights of the shoots and roots, relative water content, and total dissolved salt content of the initial leaves (cotyledons).

According to Kaveh et al. (2011), there is a substantial negative link between salinity and the rate and percentage of germination in *Solanum esculentum*. This correlation causes delayed germination and a reduced germination percentage. Abdel-Farid et al. (2020) achieved the following results: the germination rate of tomato seeds was dramatically reduced at salinity levels of 50, 100, and 200 mM NaCl and entirely inhibited at 100 and 200 mM NaCl. The authors clarified that partially osmotic or ion toxicity may have impaired enzyme activity, which could account for the delay in seed germination. When compared to the control (0 mM), González-Grande et al. (2020) observed that the tomato cultivar Río Grande's seed germination rate decreased by 6.4% at 85 mM NaCl. The rate of germination was less than 2.8% at 171 and 257 mM NaCl, indicating a significant impact on the process.

Ahmed et al. (2022), in a study about five levels of salinity and four varieties of tomato (Binatomato-6, Binatomato-7, Binatomato-8, and Binatomato-9), found that after 11 days of sowing the seeds, the highest percentage of seed germination (81.33%) of tomato was recorded in the case of the control (0 mM NaCl) and the lowest percentage of seed germination (0%) was observed in the case of the 150 mM NaCl salt solution.

According to Adilu & Gebre (2021), a decline in the water potential gradient between seeds and the medium they are growing in can be the cause of the decrease in germination rate and percentage under salt stress. Moreover, gibberellin acid concentration and enzyme activation during seed germination are impacted by the osmotic and toxic effects of NaCl. In comparison to the untreated control condition, Chakma et al. (2019) found that the percentage germination, germination coefficient, radicle length, seedling vigour index, fresh weight of radicle, mean germination time, and germination index reduced as the salt concentration increased. Al-Harbi et al. (2008) demonstrated that for all cultivars, the germination percentage was higher and the germination rate was faster at the lowest level of salinity (0.5 dS m^{-1}). The final germination percentage decreased, and the germination rate was delayed as salinity increased. In a lab experiment, Mustafa et al. (2021) showed that salinity at 12 dS m^{-1} considerably lowered all indicators compared to salinity at 6 dS m^{-1}. The germination percentage, speed, mean germination time, mean daily germination, peak value of germination, and germination value were all decreased by 73.45%, 72.59%, 28.94%, and 87.58%, respectively, as compared to the control when salinity was applied at a 12 dS m^{-1} level. The highest salinity level (5 dS m^{-1} NaCl) resulted in the lowest germination percentage (11.67%) for variety Eshet. Increasing salinity levels from 1 to 5 dS m^{-1} NaCl significantly reduced the standard germination percentages compared with the control (Shamil et al., 2020).

Plant morphology can be affected by salt at any stage of growth. Variations may be seen in the height of the plant, the ratio of roots to shoots, the size of the leaves, the number of branches, or the quantity of leaves or flowers per plant. Research on the impact of salinity on tomato plants revealed that the degree of morphological alterations in plants is influenced by the salinity of the growing medium.

The first response of plants to salinity is to reduce leaf area, which leads to reduced growth. Based on the findings of Sardoei and Mohammadi's (2014) research, it can be inferred that root and shoot growth indices were the ones that were most rapidly impacted by salt stress. When Sanjuan et al. (2015) tested 48 native tomato lines and commercial controls in Mexico at five different electrical conductivity (EC) levels using NaCl in the nutrient solution, they discovered that salt decreased the number of leaves, stem diameter, leaf area, and plant height. Moreover, the Salinity Susceptibility Index (SSI $\leq$ 1) indicated that 75% of the materials assessed were tolerant to salinity. Albacete et al. (2008) found that tomato (*Solanum lycopersicum* L.) root fresh weight decreased up to 30% when exposed to 100 mM NaCl of salt stress. According to Kakar et al. (2019), salt stress adversely influenced the tomato plant growth rate and development, as well as the number of fruits, fruit weight, leaf number, leaf area index, fresh weight, dry weight, and maximum height of the tomato plant at harvesting. Additionally, Romero-Aranda et al. (2001) reported data indicating that salinity inhibits the expansion of leaves in two tomato varieties. Common findings showed that the tomato plant's leaf area index decreased when exposed to salt stress at concentrations of 40 and 60 mM (Hajiaghaei-Kamrani et al., 2013). Furthermore, the study of variability revealed that, in comparison to other treatments, salinity at 12 dS m^{-1} considerably decreased all growth indices, with the exception of the fresh and dry weight of the shoot, which didn't differ significantly from salinity at 6 dS m^{-1}. According to Mustafa et al. (2021), the salt treatment with 12 dS m^{-1} decreased the plant height, leaf area, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight when compared to the control treatment.

Tomato dry weight, leaf area, plant stem, and roots decreased when salt exceeded 4000 ppm (Omar et al., 1982). Al-Rawahy (1989) clarified that a combination of the osmotic and particular ion actions of Cl and Na may cause the reduction of dry weights brought on by elevated salinity.

The effect of salinity on tomato physiology

Plants under salinity stress have lower water potential or content, which promotes the closing of stomatal pores, preventing more water loss through transpiration (Zhang et al., 2009). In addition to reducing transpiration because of stomatal closure, salt stress lowers net photosynthesis by lowering chlorophyll content (Flexas et al., 2007; Zribi et al., 2009). Chlorophyll content (Chl) was shown to be strongly impacted by the NaCl and drought treatments. Plants grown at 150 mg NaCl and drought + 150 mM NaCl had total Chl levels that reduced from 12.8 mg/g^{-1} f.w. in the control to 10.9 mg/g^{-1} f.w. and 7.0 mg/g^{-1} f.w., respectively (Giannakoula & Ilias, 2013). Some varieties that were exposed to 200 mM NaCl showed signs of necrosis and wilting in their leaves after one week of NaCl treatments; other genotypes took longer than two weeks to reach these stages (at 150 or 200 mM NaCl); still other varieties just dried up and died in less than a week (Murillo-Amador et al., 2017).

Salinity reduces the concentration of photosynthetic pigments and, as a result of diffusion constraints, lowers CO_2 availability, which reduces tomato physiological efficiency (Ashraf and Harris, 2013). Habibi et al. (2019) observed that salinity treatments (50, 100, 150, and 200 mM) decreased plant height, root length, the number of flowers, photosynthetic rate, transpiration rate, and stomatal conductance, but increased leaf temperature.

Increasing salt levels have been linked in several studies to decreased chlorophyll content and features related to photosynthesis (Raza et al., 2017; Gharbi et al., 2018; Kalaji et al., 2018), increased proline accumulation (Gharsallah et al., 2016; Parvin et al., 2019), antioxidant metabolism activities (Soares et al., 2019; Parvin et al., 2019; Abdelaal et al., 2019), and K^+/Na^+ ratio (Raza et al., 2017; Ishikawa & Shabala, 2019).

In addition, salinity also represses the development of leaf, flower, and fruit by slowing cell division or elongation, hindering sugar metabolism, and reducing water input, respectively (Siddiqui et al., 2017; Pinedo-Guerrero et al., 2020). Moreover, Zhang et al. (2016) demonstrate that high concentrations also reduce leaf chlorophyll content, stomatal resistance, and photosynthetic activities. In an experiment carried out in Egypt during 2020-2021, it was demonstrated that salinity increased specific leaf weight, osmotic pressure in leaves, water use efficiency (WUE), leaf total sugar content, leaf proline content, and electrolyte leakage in leaves (Abdalla Hassan Radwan et al., 2023). As reported by Tomescu et al. (2017), all plants that were watered with saline solution showed a substantial reduction in the photosynthetic outcomes (assimilation rate and stomata water vapour conductance).

According to El-Hendaway et al. (2005), salinity can significantly reduce photosynthesis and increase transpiration rate, which leaves the growing organs with insufficient absorption and ultimately delays or stops growth. Furthermore, the physicochemical characteristics of planting soil may be negatively impacted by the addition of NaCl solution (Fontes & Ronchi, 2002). Due to the toxicity of salt and chloride ions at high concentrations in the plant and the reduction of soil moisture availability, salinity has a negative impact on crop plant output (Mallick et al., 2020).

The effect of salinity on tomato yield and quality

When soluble salts are present in the soil in "excessive" amounts or concentrations, whether naturally occurring or as a result of improperly managed irrigation water, salinity becomes a concern. Most of the research carried out with tomatoes suggested a positive or no impact of salinity on fruit quality. Research on tomato landraces showed that salt enhances the flavonoids and sugar content of the fruit (Moles et al., 2019). In addition, Hurtado-Salazar et al. (2018) showed that high levels of salinity and water stress favor the increase of soluble solids in cherry tomato fruits, with a decrease in production as abiotic stress increases. Saito et al. (2008) demonstrated that moderate salt stress (EC 8.0 dS m^{-1}) enhances the accumulation of citric and malic acids by 1.7- and 2.5-fold, respectively, compared to control conditions (EC 2.5 dS m^{-1}) at the red-ripe stage. In a study, Azarmi et al. (2010) demonstrated that salinity enhanced qualitative attributes. Above 3 dS m^{-1} EC, there was a significant ($P \leq 0.05$) increase in total soluble solid. Fruit dry weight rose by 8.7% at an EC of 6 dS m^{-1} over 2.5 dS m^{-1}. When compared to 2.5 dS m^{-1}, titratable acidity increased by 2.7, 9.9, 20.3, and 28.9% at EC of 3, 4, 5, and 6 dS m^{-1}, respectively. In accordance with Liu et al. (2014), tomato cultivars of Tainan ASVEG No. 19, and Taiwan Seed ASVEG No. 22 had greater percentages of total soluble solids (16.3%, and 50%) and titratable acid (50%, and 45.3%) under the 150 mM NaCl stress condition compared to those under the 0 mM NaCl condition. On the other hand, when a 200 mM NaCl solution was used as irrigation water, there were observed losses in the dry matter of 61% in leaves, 40% in stems, and 44% in roots (Zribi et al., 2009).

Zhang et al. (2016) also confirmed that both the total fruit sugar and total acid content of tomatoes increased as salinity increased; additionally, raising the salinity of the nutrient solution from 0.78 dS m^{-1} to 1.58 dS m^{-1} resulted in an increase in sugar and acid content to 14.3% and 28%, respectively.

Many research studies have established that excessive salt concentrations lead to metabolic imbalances that reduce plant productivity (Kusvuran et al., 2016). Total yield of tomato is significantly reduced at salinity equal to and above 5 dS m^{-1}, with a 7.2% yield reduction per unit increase in salinity (Zhang et al., 2016). Abdalla Hassan Radwan et al. (2023) demonstrated that salinity increased nonmarketable yield and fruit contents of TSS, Vit. C, TA, and fruit firmness. Also, salinity enhanced Na and Cl contents in both young and old leaves, but their concentrations were higher in older leaves than in younger ones.

Also, experiments have demonstrated that the salinity of irrigation water may result in a decrease in tomato output, particularly if the applied water's salt content is higher than the salt tolerance threshold (Kang et al., 2010, Li et al., 2019, Li et al., 2022). In 2021, Ladewig et al. examined the responses of four tomato landraces and one hybrid to four different NaCl concentrations (0, 30, 60, and 90 mM). When comparing plants exposed to 30, 60, and 90 mM to the control, they found that the cultivar "Campeche" performed lowest in response to salinity, showing the most significant yield reductions of 71.1%, 80.1%, and 89.6%, respectively. Del Amor et al. (2001) demonstrated that salinity reduces the growth rate of tomato (*Lycopersicon esculentum* Mill. cv. Daniela), primarily the number of fruits and also fruit size. Magán et al. (2008) found that tomato fresh yield is significantly decreased by salinity. Siddiky (2012) & Siddiky et al. (2014) revealed that there was a notable difference in fruit weight among various tomato germplasms and that plants treated with high concentrations of salt generally showed a significant loss in fruit yield when compared to controls. In a study conducted in Bangladesh, a significant decrease in tomato yield was found in all five studied BARI-T (1–5) varieties at 6 and 8 dS/m^{-1} salinity levels. A reduction in fruit number of 2.0% was reported with an increase of 1 $dS\ m^{-1}$ beyond the threshold value of 4.4 $dS\ m^{-1}$ (Magán et al., 2008). Sharma & Sachan (2023) noted that the application of a saltwater concentration at EC 8 $dS\ m^{-1}$ produced the lowest fruit yield of 145.59 g, while the application of no saltwater concentration (control) produced the highest fruit yield of 323.52 g. As salinity increased, there was a significant reduction in fruit output. The findings of Mou (2021), El-Mogy et al. (2018), Islam et al. (2011), and Mazumder (2016 a and b) were in agreement with this result. Zhang et al. (2022) found that tomatoes produced at salinities of 5 $dS\ m^{-1}$ and higher had a significantly lower total yield. Moreover, Ahmad et al. (2017) also showed that the yield of tomato cultivars (Pearl and MT1) was affected by salinity, reducing their productions by 84% at the highest concentration of salinity. Even at the medium level of NaCl (70 mM), both of the cultivars had lost 62% of their yield, indicating a moderate salinity-tolerance of the cultivar studied.

CONCLUSIONS

Salinity is one of the harshest environmental variables limiting crop productivity. Studying plant growth throughout the course of the growing season can reveal information about a crop's long-term salt tolerance. This review indicates that tomato varieties are more vulnerable to the adverse effects of salt in the form of NaCl solution during the seedling stage of growth than they are later in the life cycle. The study also highlights the fact that, although some salinity treatments have been shown to improve tomato quality, there have been reports of adverse effects of increased salinity on tomato yield.

Based on the prediction that over 50% of arable land will turn salty by 2050, researchers ought to concentrate more on finding techniques to desalinate soils, studying how to create fertigation plans that encourage better management of water and fertiliser applied in accordance with plant requirements, developing new salinity-resistant varieties, or enhancing already existing species.

However, further field research in salt-affected soils is needed to get a better understanding of how salinity affects tomato plants. This research should take into account the individual and cumulative interactions of the many components.

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THE INFLUENCE OF CULTIVAR AND ORGANIC FERTILIZATIONS ON PLANT GROWTH, PRODUCTION AND QUALITY OF SWISS CHARD, IN WESTERN ROMANIA

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Abstract

Swiss chard (Beta vulgaris L., ssp. cicla) are grown for whole leaves or only for petiole. In Romania, chard is a less cultivated species, being present in some areas of Transylvania. Research aim was to establish how some Swiss chard cultivars behave, after fertilization with three different organic fertilizers. Plant growth, total production as well as dry matter, phenols, flavonoids, dietary fiber, carbohydrates and vitamin C content was measured. Experiment took place between 2021-2022, in an organic vegetable farm, in Săcueni, Bihor county, Romania. Three organic fertilizers were used: Lignohumate, Alcygol Z2M and Alg Green, applied 30 days after emergence, at the dose recommended by the manufacturer. Plant development, 60 days after emergence, was higher at Lucullus cultivar, fertilized with Alg Green, 54.35 cm. Production ranged from 44.67 t/ha (Carde Blanche d'Ampuis fertilized with Lignohumate) to 51.04 t/ha (Lucullus fertilized with Alg Green). Plant content in dry matter, fibers and vitamin C, was higher when plants were fertilized with Lignohumate. Phenols and flavonoids were higher at variants fertlized with Alg Green.

***Key words**: chemical composition, organic fertlizers, plant development, swiss chard.*

INTRODUCTION

Swiss chard (*Beta vulgaris* L.*,* ssp. *cicla*) is a dark green leafy vegetable (GLV) available throughout the year. Swiss chard could be planted in mid spring and again in late summer, which indicates the possibility of harvesting during a long period (Kolota et al., 2010). Chard is a commonly used food crop which is found along the shores of the Mediterranean, frequently used all over the world due to its nutritional value and delicious taste. Chard is a desirable food crop because it adapts to environments with elevated saline concentrations, and it can grow in soils with scarce availability of water (Ninfali and Angelino, 2013). Furthermore, Swiss chard is tolerant to conditions of low light and both cold and hot weather (Kolota et al., 2010).

GLVs are exceptionally low in energy but also relatively high in micronutrients and phytochemicals, which recommend GLVs for consumption in everyday diet (van Jaarsveld et al., 2014). Certain epidemiological studies promoted consumption of GLVs because these vegetables were found to protect against numerous chronic diseases caused by free radical activity (Slavin and Lloyd, 2012). Swiss chard, as one of the GLVs, is rich in phytopigments such as chlorophyll and carotenoids, flavonoids and minerals with antioxidant and immunomodulating properties (Ivanovic et al., 2019). Phytopigments improve immune, detoxication and antioxidant systems of the human body, thus indirectly helping the prevention of disease (Fiedor and Burda, 2014). Swiss chard is a very good source of vitamins C, A and B, phenolic acids (syringic, caffeic and p-coumaric), flavonoids (kaempferol, quercetin and glycosides derived from apigenin) and minerals such as iron, potassium, calcium, magnesium and manganese, which additionally contributes to the functionality of Swiss chard (Ninfali & Angelino, 2013). Swiss chard is also rich in dietary fibers, proteins and antioxidants such as alpha-lipoic acid, which is linked to lower glucose levels and increased insulin sensitivity (Ivanovic et al., 2019; Yang et al., 2014).

The plant has a thick, crunchy stalk that can be white or colorful and wide fanlike green leaves (Rana, 2016). Leaves can be consumed raw as part of a salad or cooked alone or along with the stems in a similar way as spinach (Dietitians of Canada, 2020).

In Romania, chard is a less cultivated species, being present in some areas of Transylvania but not in organic culture. Organic farming is an important and ever-growing sector of agriculture and of Romanian economy, because it can bring significant contribution to a sustainable development, increasing the economic activities, thanks to the significant added value of the organic products (Saracin & Vasile, 2015).

Factors such as climate, environmental conditions, applications of fertilizers, time of harvesting, germination, plant physiology state, all affect nutritional properties and phytochemical content of the food crops (Lombardo et al., 2017; Miceli & Miceli, 2014). The application of the optimal amounts of nitrogen fertilizers is a common farmer practice that aims to maximize the economic return and maintain environmental quality (Miceli & Miceli, 2014). The treatment of Swiss chard with different amounts of fertilizer impacted certain nutritive factors (Petrova & Mitova, 2023) but the data regarding the effects of fertilization on phytochemical content of Swiss chard are scarce.

MATERIALS AND METHODS

Experiment took place between 2021-2022, in a organic vegetable farm, in Săcueni (47.347116, 22.099245), Bihor county, Romania. In 2021, average annual temperature was 11.5^{0}C and in 2022, 12.2^{0}C. Annual rainfall recorded was 539.21 mm in 2021, respectively 516.36 mm in 2022, according to data recorded at the Săcuieni Meteorological Station. Soil on which the experiment was placed was alluvial, with a morphological profile of the Ao-AC-CN type. From a physical point of view, the soil in the experimental area has the following characteristics: clay content (0.002 mm) 16.4-19.1; total porosity value, large-51; low apparent density, 1.25 g/cmc; permeability per profile, high - 18 mm/h; useful edaphic volume, large-100. Chemically, the soil had the following characteristics: soil reaction, weakly acidic, pH 5.95-6.4, throughout the profile; humus content, small 1.14-1.51; total nitrogen content, low 0.075; mobile phosphorus content, small 12 ppm; mobile potassium content, very low 60 ppm.

Three organic fertilizers were used: Lignohumate (dose of 1 kg/ha), Alcygol Z2M (dose of 5 l/ha) and Alg Green (dose of 1 l/ha), applied 30 days after emergence. Control remained unfertilized. Three Swiss chard cultivars were tested, Lucullus, Carde Blanche d'Ampuis and Verca.

Establishment of chard culture was carried out by direct sowing on April 28 (2021) and 29 (2022). By combining the two experimental factors (cultivar x fertilizer used) resulted in 9 experimental variants, which were placed in three repetitions. Plot size was 9 m^2 (3 m × 3 m). Chard was planted into rows spaced 25 cm apart.

During the growing season, development degree and plant growth (plant height, leaf rosette diameter, number of leaves, leaf length, length and thickness of petiole) and production (total production, production of petioles) were determined. After harvesting, determinations of dry matter dry matter, betalains, flavonoids, dietary fiber, carbohydrates and vitamin C. Average data of the experimental years 2021 and 2022 is presented in this manuscript.

The edible parts of Swiss chard, leaves and steams were washed with tap water and double distilled water. They were drained completely, dried over filter paper and homogenized with domestic processor in a dark room at 25°C. Vitamin C was determined after the homogenization of fresh Swiss chard. The rest of the samples were packaged in a vacuum plastic bag and stored at −25°C until analysis.

Moisture content was determined by drying the edible parts of Swiss chard in an oven at 105°C until constant weight was obtained. The samples were incinerated in a muffle furnace at 520°C until constant weight was obtained in order to evaluate the ash content (Latimer, 2012). Dietary fibers were determined according to the Scharrer-Kurscher method (Matissek & Steiner, 2006). Total carbohydrates were calculated by subtracting the total amount of proteins, total lipids, total ash, moisture content and dietary indigestible fibers out of a hundred (Ivanovic et al., 2019).

The content of vitamin C in Swiss chard samples was measured on a Reflectometer RQflex10 Reflectoquant using Reflectoquant ascorbic acid test strips (Reflectoquant® Ascorbic Acid Test, Merck, 2016). The results were presented as mg of vitamin C in 100 g FW.

Total flavonoid content (TFC) was determined, according to the method of Sakanaka et al. (2005), using catechin as a standard flavonoid compound. A 0.5 mL of the chard extract (1 mg/mL) was taken in a test tube and 2.50 mL of distilled water and 0.15 mL of a 5 percent $NaNO_2$ solution were added. After 6 min, 0.3 mL of a 10 percent $AlCl_3$ solution was added and allowed to stand at room temperature for 5 min and then 1 mL of 1M NaOH was added to the test tube. The solution was then diluted with distilled water to make the final volume up to 5 mL. The absorbance was read at 510 nm. TFC was calculated from a calibration curve, and the result was expressed as mg of CAE per mg of the water extract. The absorbances were measured using a spectrophotometer (Varian Cary 3E UV-VIS).

Chard soluble carbohydrates were extracted based on the method described by Bartolozzi et al. in 1997. The extract was dried and transformed into trimethylsilyl ethers by treatment with pyridine, hexamethyldisilazane and trimethylchlorosilane. Soluble sugars were analyzed using a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS column (30 m x 0.25 mm). The soluble sugars contents were expressed as g per 100 g of fresh weight (fw).

Total flavonoids content was determined according to (Zhishen, Mengcheng, & Jianming, 1999). An aliquot (0.5 mL) of Swiss chard solution was mixed with distilled water (2 mL) and subsequently with a $NaNO_2$ solution (5%, 0.15 mL). After 6 min, an $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min. After wards, a NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to adjust the final volume to 5 mL. Then, the blend was properly mixed and allowed to stand for 15 min. The intensity of the pink color was measured at 510 nm. (+)-Catechin was employed to prepare the standard curve and outcomes were expressed as mg of (+)-catechin equivalents (CE) per g of chard extract.

Total phenolics content was determined based on Stojovic method (Stojković et al., 2014). In fact, an aliquot of the chard ethanol extract (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL). The tubes of appropriate dilutions of the ethanol extract were homogenized for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then read at 765 nm. Gallic acid was employed as standard for the calibration curve, and results were expressed as mg of gallic acid equivalents (GAE) per g of chard extract. Results were expressed as a mean ± SD. Significant differences between the means, for experimental variants, were determined with two-way ANOVA and Duncan's test at $p < 0.05$.

RESULTS AND DISCUSSIONS

Growth parameters (plant height, petiole lenght and petiole width) of Swiss chard are presented in Table 1. Plant nutrition through application of fertilizers can influence growth, plant morphology, yield and quality (Ullah et al., 2016). Growth of chard plants, 30 days after emergence (DAE), averaged between 18.33 cm at Verca and 19.12 at Carde Blanche d'Ampuis. This measurement was performed before the application of organic fertilizers. Swiss chard plant growth was influenced by the fertilization with organic fertilizers. Unfertilized variants recorded lower values compared to fertilized variants at all studied cultivars. Regarding plant height 60 DAE, the highest value of 54.35 cm was registered at Lucullus fertilized with Alg. Green. Pokluda and Kuben (2002) determined plant height in 13 Swiss chard cultivars where values ranged from 42.5 cm (Charlote) to 57.9 cm (Swiss Chard-Kings).

Table 1. Influence of cultivar and organic fertilizers on plant growth (average for 2021-2022)

Variant		Plant height (cm) after:		Petiole length (cm)	Petiole width (cm)
Cultivar	Fertilizer	30 days	60 days	60 days	60 days
Lucullus	Unfertilized	18.77 d	48.67 J	13.42 f	2.03 i
	Lignohumate	18.81 c	53.22 B	14.81 c	3.12 f
	Alcygol Z2M	18.73 d	49.61 G	14.53 d	2.72 h
	Alg Green	18.72 d	54.35 A	15.83 a	4.05 b
Carde Blanche d'Ampuis	Unfertilized	18.65 e	49.00 J	13.54 f	2.33 i
	Lignohumate	18.71 d	51.52 F	15.26 b	4.24 a
	Alcygol Z2M	19.12 a	52.81 C	14.91 c	3.03 g
	Alg Green	18.55 f	49.25 I	15.35 b	4.02 b
Verca	Unfertilized	18.46 g	48.53 J	12.52 f	2.54 i
	Lignohumate	18.43 g	51.82 E	14.91 c	3.71 d
	Alcygol Z2M	18.63 e	49.41 H	14.22 e	3.31 e
	Alg Green	18.94 b	52.05 D	15.31 b	3.92 c
LSD P = .05		0.065	0.090	0.206	0.094
Standard Deviation		0.045	0.062	0.142	0.065

Means followed by same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)

Petiole length 60 DAE, the highest value of 15.8 cm was registered also at Lucullus fertilized with Alg. Green. Petiole width 60 DAE was between 2.03 cm at unfertilized Lucullus variant and 4.24 at Carde Blanche d'Ampuis fertilized with Lignohumate. Similar results were obtained by Kolota et al., in 2017, when they studied Swiss chard Lucullus plants development under the effect of different doses of nitrogen.

Compared with the average of unfertilized variants, results obtained by fertilized variants were statically assured regarding al measured traits.

Swiss chard harvesting began in the second decade of July and continued until the second decade of November (in both years), with 6 harvests carried out during the growing season. Volume of harvested production increased from the beginning of the harvest period until September, after which it decreased to all experimental variants. Lowest yields were obtained by unfertilized variants. For the fertilized variants, yields varied between 44.67 t/ha at Carde Blanche d'Ampuis cultivar fertilized with Lignohumate and 51.04 t/ha at Lucullus fertilized with Alg Green (Figure 1). Compared to the average of unfertilized variants, the differences in production obtained in fertilized variants are significant (Figure 1). This values are simillar or higher to those obtained by Krishkova et al., 2022, in an experience with 13 cultivars, yields ranging from 16 t/ha to 52 t/ha. Lower yields, of 43.5 t/ha were recorded for Lucullus, in organic culture, by Murga-Orrillo et al., in 2019.

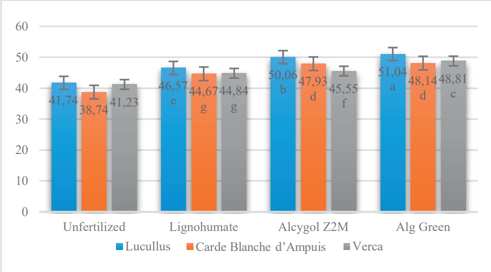

Figure 1. Swiss chard production (average 2021-2022)
Means followed by same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)
LSD P = .05 0.601
Standard Deviation 0.414

Dry matter (DM) content was measured at each harvest and averages were made for the summer and autumn months. DM content was lower in unfertilized variants compared to fertilized ones, at all cultivars. For harvests done in summer, DM content of leaf petiole averaged between 7.05% for Lucullus unfertilized and 9.05% for Verca fertilized with Lignohumate (Table 2). In leaf blades, DM content was higher compared to values recorded in petiole, so that during summer harvests, values were on average between 11.88% (Lucullus unfertilized) and 13.90% (Verca fertilized with Lignohumate). For autumn harvests, DM content was higher, in leaf petiole and leaf blades compared to summer harvests. Bozokalfa et al., 2016, determined DM content of 52 Swiss chard cultivars, minimum value being 9.02%, maximum value being 18.53% and average was 10.89%. In similar experiments, Lucullus, Green White, Ribbed, Vulcan, Bresanne, Green Silver varieties had on average a DM content in leaf blades of 11.62% in summer harvested plants and 13.96% in autumn. In the leaf petiole the content was 6.93% for summer harvested plants and 8.54% autumn (Kołota et al., 2010).

Table 2. Influence of cultivar and fertlizer on swiss chard plant content in dry matter and vitamin C (average for 2021-2022)

Variant		Dry matter content (%) in:				Vitamin C content (mg/100 g f.w.)			
Cultivar	Fertilizer	Leaf petiole		Leaf blades		Leaf petiole		Leaf blades	
		Summer	Autumn	Summer	Autumn	Summer	Autumn	Summer	Autumn
Lucullus	Unfertilized	7.05	8.12	11.88	13.79	33.25	48.93	23.27	36.80
	Lignohumate	7.43	8.60	12.14	14.37	36.27	52.77	24.78	38.87
	Alcygol Z2M	7.26	8.37	12.09	14.08	35.50	50.45	23.62	38.04
	Alg Green	7.40	8.55	12.25	14.20	36.02	51.16	24.56	38.51
Carde Blanche d'Ampuis	Unfertilized	8.04	9.83	12.85	14.28	37.44	58.37	23.74	37.66
	Lignohumate	8.71	10.29	13.57	15.21	39.80	61.72	25.08	39.66
	Alcygol Z2M	8.33	10.07	13.20	14.76	39.01	60.79	24.85	39.03
	Alg Green	8.65	10.34	13.38	15.12	39.36	61.12	24.70	39.21
Verca	Unfertilized	8.72	9.83	13.12	14.17	36.51	54.30	24.00	36.22
	Lignohumate	9.05	10.45	13.90	14.70	37.92	57.42	25.12	38.42
	Alcygol Z2M	8.87	10.18	13.44	14.51	36.73	56.77	24.65	37.58
	Alg Green	9.02	10.33	13.78	14.62	37.40	57.08	25.04	38.12

Vitamin C content was lower in unfertilized variants compared to fertilized ones, at all cultivars. Vitamin C content was higher during autumn harvests in both leaf petiole and blades (Table 2). Leaf petiole also contained a higher amount of vitamin C compared to leaf blades. Similar level of vitamin C concentration in this vegetable crop within a range of 43.8 and 59.8 mg/100 g f.w., depending on the organic fertilizers, was found by Rivelli & Libutti, 2022.

In this study, dietary fiber content (DF) outcomes revealed that DF (Table 3) was between 2.45 g/100 g f.w. for Carde Blanche d'Ampuis unfertilized and 2.74 g/100 g f.w. for Lucullus fertilized with Lignohumate. Similar results were obtained by Mzoughi et al., 2019. Generally, the chemical composition of plant species differs depending on the harvest period and the growth conditions (e.g. climate, soil quality, irrigation, treatments, etc.). It is well-known that a daily intake of 7 g of plant dietary fibers is considered enough to significantly decrease the menace of cardiovascular and coronary heart diseases (Barreira et al., 2017). In this sense, the consumption of 100 g of fresh wild Swiss chard leaves would cover approximately 35% of the recommended daily dose. Thus, chard leaves can be considered an interesting source of dietary fiber and could be added to other foods to improve fiber intake (Mzoughi et al., 2019).

The consumption of vegetables that are rich in phenols and flavonoids is associated with preventions of diseases caused by oxidative stress (Ballistreri et al., 2013).

Total phenol (TPC) and flavonoid (TFC) contents are shown in Table 3. TPC varied from 18.23 to 55.78 mg of GAE/mg of extract. Sacan and Yanardag (2010) measured TPC of water extract from dry Swiss chard, and they found 31.09 mg pyrocatechol equivalent/mg extract. The difference in results may be due to the use of different equivalent, where gallic acid and pyrocatehol have different reactivity with FC reagents (Everette et al., 2010). TFC ranged from 10.21 to 14.55 mg CAE/mg of extract. The used equivalent was the same as the one used by Sacan and Yanardag (2010), and the values for TFC were in agreement with this study (11.88 mg CAE/mg of extract) (Sacan and Yanardag, 2010).

Table 3. Influence of cultivar and organic fertilizers on fiber, total phenols and total flavonoids content (average for 2021-2022)

Variant		Dietary fibers (g/100g f.w.)	Total phenols (mg GAE/g extract)	Total flavonoids (mg CE/g extract	Total carbohydrates (mg/100g f.w.)
Cultivar	Fertilizer				
Lucullus	Unfertilized	2.60	21.32	11.50	5.68
	Lignohumate	2.74	35.65	12.88	5.81
	Alcygol Z2M	2.62	44.23	13.32	6.02
	Alg Green	2.56	53.44	13.89	5.96
Carde Blanche d'Ampuis	Unfertilized	2.45	18.23	10.21	5.92
	Lignohumate	2.55	29.25	12.67	6.05
	Alcygol Z2M	2.52	34.21	13.11	6.16
	Alg Green	2.50	42.89	13.20	6.00
Verca	Unfertilized	2.56	32.18	11.02	5.80
	Lignohumate	2.68	41.12	13.21	6.02
	Alcygol Z2M	2.61	44.13	14.12	6.10
	Alg Green	2.58	55.78	14.55	5.92

However, TPC and TFC in different cultivars of Swiss chard and other vegetables vary widely and they are difficult to interpret or compare to our study, because many factors have been showed to influence TPC and TFC.

Total carbohydrates content in Swiss chard, ranged from 5.68 g/100 g f.w. at Lucullus unfertilized and to 6.16 g/100 g f.w. Carde Blanche d'Ampuis fertilized with Alcygol Z2M. These values are higher compared to 3.74 g/100 g f.w. found by the U.S Department of Agriculture (USDA, 2014) and lower compared to 6.84 g/100 g f.w. found by Ivanovic et al., 2019.

CONCLUSIONS

Swiss chard can be grown organically with good results, if organic fertilizers are applied. Growth parameters and production of Swiss chard were influenced by used cultivar and type of fertilizer. Plant height, 60 DAE, was higher at Lucullus fertilized with Alg Green (54.35 cm). This was also the case for petiole length, that reached 15.83 cm. Petiole width was higher at Carde Blance d'Ampuis (4.24 cm) when fertilized with Lignohumate. Plant content in DM, fibers and vitamin C, was higher when Swiss chard was fertilized with Lignohumate, at all studied cultivars. Swiss chard petiole DM content was higher, in the material harvested during autumn compared to the one harvested in summer. Higher DM content was registered by Verca when fertilized with Lignohumate, of 10.45% in petiole and 13.90% in leaf blades. These results are also confirmed by Colonna et al. in 2016, when they had grown Swiss chard under different light intensities and at a lower light intensity (as in autumn), the DM content is higher. Vitamin C content was lower in unfertilized variants compared to fertilized ones, at all cultivars. Vitamin C content was higher during autumn harvests at Carde Blance d'Ampuis when fertilized with Lignohumate, of 61.72 mg/100 g f.w. in petiole and 39.66 mg/100 g f.w. in leaf blades. Leaf petiole also contained a higher amount of vitamin C compared to leaf blades. Swiss chard has a good DF content, 100 g of fresh wild Swiss chard leaves would cover approximately 35% of the recommended daily dose (Mzoughi et al., 2019). High DF content was register at Lucullus fertilized with Lignohumate (2.74 g/100 g f.w.).

TPC varied from 18.23 to 55.78 mg of GAE/mg of extract. TFC ranged from 10.21 to 14.55 mg CAE/mg of extract. However, TPC and TFC in different cultivars of Swiss chard and other vegetables vary widely and they are difficult to interpret or compare to our study, because many factors have been showed to influence TPC and TFC. Total carbohydrates content was between 5.68 mg/100 g f.w. at Lucullus unfertilized and 6.16 mg/100 g f.w. at Carde Blance d'Ampuis, fertilized with Alg Green. These results were somewhat average compared to the findings of other authors and was influenced by used cultivar and fertilizer.

Results proved that chard leaves have a nutritional profile suitable to be including in modern diets. Also, chard leaves exhibit a significant content of antioxidants compounds, such as flavonoids, phenolic acids.

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CHALLENGING THE UNBEATABLE: *HELICOVERPA ARMIGERA* INFESTATION IN *MOMORDICA CHARANTIA* - A NOVEL CASE STUDY

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Abstract

Momordica charantia, commonly known as bitter gourd or bitter lemon, belongs to the Cucurbitaceae family and is a versatile herb cultivated in various tropical and subtropical regions. Despite its distinctive appearance and bitter taste, it stands out as one of the most nutritious gourds. Numerous studies have highlighted its antimicrobial properties against soilborne pathogens and inhibitory effects on human pathogens. Additionally, the plant contains compounds known to repel insect pests. In Romania, M. charantia has been successfully acclimatized at the Vegetable Research and Development Station in Buzău, leading to the development of two cultivars: Rodeo and Brâncuși. Over a span of fifteen years, this plant has demonstrated resilience, remaining free from diseases and pests that could compromise its yield. However, during the vegetation period of 2023, a notable exception occurred as researchers identified an infestation of Helicoverpa armigera in the fruit crop. Intriguingly, this pest has not been previously reported in association with bitter gourd worldwide. The existing literature does contain studies on the inhibitory effects of M. charantia compounds against H. armigera, but this case study demands further investigation.

Key words: *bitter gourd, cotton bollworm, Cucurbitaceae.*

INTRODUCTION

Bitter melon, known by various names such as bitter cucumber, bitter gourd, and karela, is a unique member of the Cucurbitaceae family, closely related to squash and cucumbers. Despite its less-than-appealing appearance and bitter taste, it stands out as one of the most nutritious and versatile vegetables, widely consumed in Southeast Asia, Indo-China (Poolperm and Jiraungkoorskul, 2017), and even in Brazil (Magalhães et al., 2019) for both culinary and traditional medicinal purposes. However, in the Western world (Basch et al., 2003), especially in countries like Romania, bitter melon dishes are not as renowned due to its distinctive taste.

The scientific name of bitter melon is *Momordica charantia* L. Delving into the etymology of the term "*momordica*", we discover its Latin origin, "*mordicus*", meaning "*biting*" (Afsar et al., 2022; Newton, 2023). This nomenclature intriguingly aligns with the grooved edges of its seeds, creating an appearance reminiscent of having been chewed. Despite its unpalatable taste, bitter melon has gained attention for its rich diversity of primary and secondary metabolites, showcasing therapeutic potential across various domains (Supe and Daniel, 2015; Bukhari et al., 2019). This unassuming vegetable proves to be a powerhouse with antiulcer properties (Gupta et al., 2011; Jia et al., 2015), antioxidant capabilities (Mala et al., 2017; Svobodova et al., 2017; Wang et al., 2017; Shafie et al., 2018), antimicrobial properties (Jia et al., 2017; Shafie et al., 2018; Zubair et al., 2018; Bukhari et al., 2019), anthelmintic prowess (Swarna and Ravindhran, 2012; Wang et al., 2016), antidiabetic potential (Jia et al., 2017; Wang et al., 2017; Peter et al., 2018; Lucas et al., 2010), anti-inflammatory attributes (Lucas et al., 2010; Peter et al., 2018), antihyperglycemic effects (Palamthodi and Lele, 2014; Jia et al., 2017; Peter et al., 2018), anticancer properties (Ji et al., 2010; Lucas et al., 2010; Wang et al., 2016; Poolperm and Jiraungkoorskul, 2017), and nutritional perks as an antilipolytic agent (Nerurkar et al., 2010; Wang et al., 2017).

The increasing global concern over bacterial resistance, predicted to be a leading cause of death by 2050 (de Kraker et al., 2016; Boklage and Lehmkuhl, 2018; Sanchez and Gustot, 2019), has thrust the spotlight on bitter melon.

In the face of bacteria becoming resistant to almost all existing antibacterials (McCarthy, 2017), the quest for novel antibacterial entities is now a global research focus (Tacconelli et al., 2018), and *M. charantia* emerges as a species holding tremendous potential. Multiple studies have highlighted its antifungal and antibacterial activity (Adeyemi et al., 2015; Supe and Daniel, 2015; Yaldiz et al., 2015), not only in the leaves (Makhija et al., 2011; Supraja and Usha, 2013; Brandão et al., 2016; Bukhari et al., 2019) but also in the fruit (Mwambete, 2009).

In various studies, *Momordica charantia* has demonstrated its potential in insecticidal prowess against a range of pests. Notably, it has exhibited effectiveness against mustard sawfly (Kumar et al., 1979), mosquitoes (Singh et al., 2006; Maurya et al., 2009; Mituiassu et al., 2021; Subramaniam et al., 2021), and common weevils, including the mung bean weevil and cowpea weevil (*Callosobruchus chinensis*; *C. maculatus*) (Ajayi, 2005; Wahyutami and Aisyah, 2022). Moreover, *Momordica charantia* has displayed insecticidal properties against aphids such as sugar cane aphids (*Melanaphis sacchari*) and mustard aphids (*Lipaphis erysimi*) (Mishra et al., 2006; Salinas-Sánchez et al., 2021).

While it's generally believed that bitter gourd's toxic compounds make it resistant to foliage pests and diseases (Robinson and Decker-Walters, 1997), our observations reveal a novel challenge - an infestation with *Helicoverpa armigera* (Hübner), commonly known as the cotton bollworm. This unexpected occurrence prompts a reassessment of bitter melon's pest resilience and introduces a new dimension to its cultivation considerations.

MATERIALS AND METHODS

The Vegetable Research Development Station in Buzau, Romania, stands as a prominent research center dedicated to the acclimatization of new plant species. Notably, since the early 2000, the research center has undertaken an acclimatization program focused on *Momordica charantia*, commonly known as bitter melon. As a result, the VRDS portfolio proudly boasts two commercially available varieties of bitter melon named Rodeo and Brâncuși (Figures 1, 2).

Figure 1. Bitter melon cultivar: Brâncuși

Figure 2. *Momordica charantia* cultivar: Rodeo

Momordica charantia is an annual, monoecious climbing plant with a cardinal taproot. The stems extend from the top of the taproot, spreading to climb any available support, necessitating the use of a trellising system. The plant features modest leaves, measuring 4-6 cm in width, with 3-7 deeply separated lobes. The pendulous fruits, characterized by 2-9 cm long stalks, typically exhibit a spindle or ellipsoid shape, appearing warty or ridged. They dehisce unevenly, resembling a 3-valved fleshy capsule. In terms of colour, the Brâncuși variety displays a white-green hue when immature, while the Rodeo cultivar takes on a dark green colour during this stage. Additionally, the Brâncuși fruit can attain lengths of up to 40 cm, whereas the Rodeo variety produces smaller fruit ranging from 10-15 cm. Notably, the Rodeo variant features more prominent ribs compared to the Brâncuși cultivar.

For the cultivation process, the cultivars were sown in the first decade of March and transplanted in the last decade of April in polytunnels. The selected planting scheme maintained a spacing of 70 cm between plants and 150 cm between rows. The obtained yield was 1.2 tons per 400 square meters. Throughout the vegetation period of 2023, diligent monitoring efforts were employed to assess and combat potential pathogen attacks. Pathogen attacks were quantified using key indicators, including the frequency of attack (F%), intensity of attack (I%), and the degree of attack (DA%).

RESULTS AND DISCUSSIONS

Bitter gourd faces several challenges from insect pests and diseases, although none of them have been deemed significantly impactful (Robinson and Decker-Walters, 1997). In the relevant literature, the primary pests of bitter melon are typically recognized as the ladybird beetle (*Epilachna septima*), fruit borer (*Diaphania indica*), fruit fly (*Bactrocera cucurbitiae*), and red pumpkin beetle (*Aulacophora foveicollis*) (Bharathi and John, 2013). However, these pests are predominantly prevalent in Asia and Africa, not in Europe. In the European context, bitter melon cultivation may be prone to interference from the leaf miner (*Liriomyza trifolii*) and the root-knot nematode (*Meloidogyne incognita*).
In Romania, bitter gourd has exhibited tolerance to specific pathogenic attacks (Lagunovschi-Luchian et al., 2017; Bute et al., 2020). Our study aligns with this trend, as we observed minimal interference from the leaf miner, with the primary interaction being with *Meloidogyne incognita*. Although the frequency of attack occurred over 33.7% of the crop surface, and the intensity of the attack ranged from mild to medium, it did not result in significant yield losses (Figure 3).

Figure 3. Nematode infestation on *M. charantia* roots

Disease resistance is a crucial trait in bitter gourd cultivation due to its potential to significantly reduce both yield and quality. The escalating use of chemicals for pest and disease management raises apprehensions among growers about both economic viability and human health (Kole et al., 2020).
Globally, *Fusarium* wilt (*Fusarium oxysporum* f. *niveum*) is the most prevalent disease in bitter gourd crops. *Fusarium* wilt, caused by *Fusarium oxysporum*, is a common and destructive soil-borne disease affecting cucurbit crops worldwide. Additionally, anthracnose (*Colletotrichum lagenarium*), powdery mildew (*Sphaerotheca fuliginea*), downy mildew (*Pseudoperonospora cubensis*), and fruit rot (*Pythium aphanidermatum*) contribute to significant foliar and yield losses in bitter melon crops (Bharathi and John, 2013). In Romania, sporadic anthracnose attacks were observed, with a frequency of 35.7%, intensity at 10%, and a degree of attack at 3.6% (Bute et al., 2020). At VRDS Buzau, no diseases affecting yield or fruit quality have been recorded.
The atypical weather patterns of 2023, amidst the broader context of climate change, witnessed heatwaves during the summer leading to an increase in pest activity, notably *Helicoverpa armigera*, commonly known as the cotton bollworm. Towards the end of August 2023, sporadic attacks on bitter melon fruits by *Helicoverpa armigera* were noted after a period of pest-free conditions (Figures 4 and 5).

Figure 4: *Helicoverpa armigera* infestation on bitter melon fruit

The sporadic infestations noted towards the end of August 2023 were attributed to the third to sixth instar of the second-generation larvae of *Helicoverpa armigera*. Regarding the established indicators frequency of attack (F%), intensity of attack (I%), and the degree of attack (DA%) these metrics were determined based on the activities of the aforementioned third to sixth instar larvae of *Helicoverpa armigera*.

Figure 5. Bitter melon fruit and cross-section under *Helicoverpa armigera* attack

The frequency of attack was recorded at 12.3%, with variations in intensity observed among different cultivars. Notably, Brâncuși exhibited higher susceptibility (7.1%) compared to Rodeo (5.48%). The degree of attack ranged from 6.4% (Rodeo) to 8.6% (Brâncuși).

It is crucial to emphasize that these indicators provide insights into the behavior and impact of the specific generation of *Helicoverpa armigera* larvae under investigation. Importantly, the cotton bollworm demonstrated a distinctive trait by exclusively targeting the fruits, leaving the leaves unaffected.

Despite the unexpected nature of these occurrences, the persistence, virulence, and ongoing impact of the attacks warrant further study in subsequent years. This exploration aims to discern whether the observed trend in 2023 was a singular event influenced by the heatwaves or indicative of a continuing pattern.

Worldwide, *M. charantia* is extensively studied for its undeniable benefits. In Romania, while this plant is not as common, recent studies are increasingly recommending its cultivation for both crops and medicinal purposes.

Notably, in Romania, pests and diseases do not pose a significant threat, emphasizing the importance of maintaining the crop's current status.

To address potential pest and disease issues in *Momordica* cultivation in Romania, it is crucial to implement thorough crop monitoring and utilize cultivars with high tolerance. Moreover, imposing restrictions on the import of fruits susceptible to various plant pathogens and diseases becomes essential. It is worth noting that the European Union has established specific import requirements for bitter melon fruits from Sri Lanka, Thailand, Honduras, and Mexico. These requirements emphasize the necessity for produce originating from pest-free areas or those with comprehensive physical protection. The stipulations also highlight the importance of implementing multiple measures throughout the production, handling, and inspection processes (Official Journal of the European Union, 2022).

In Romania, we have *Momordica* cultivars, including Brâncuși and Rodeo, renowned for their robust yield potential and excellent adaptation to the local pedoclimatic conditions. The cultivation of these varieties not only proves to be viable but also offers substantial advantages for farmers.

CONCLUSIONS

Momordica charantia, though less common in Romania, has been gaining attention for both agricultural and medicinal purposes. Pest and disease management, particularly in the face of an emerging threat from *Helicoverpa armigera*, underscores the need for vigilant crop monitoring and the adoption of tolerant cultivars. Notably, local cultivars like Brâncuși and Rodeo showcase high yield potential and adaptability to the local pedoclimatic conditions, making them not only viable, but also advantageous choices for Romanian farmers. The impact of climate change, exemplified by heatwaves in 2023, further emphasizes the importance of ongoing research to understand and address evolving challenges in *Momordica* cultivation.

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ELEMENTS OF PRODUCTIVITY IN TOMATO PLANTS IN RELATION TO FOLIAR FERTILIZATION

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Abstract

The study evaluated productivity elements in tomato plants in relation to foliar fertilization. 'Beldine F1', 'Sandoline F1', 'Ciciu F1' and 'Izmir F1' tomato hybrids were grown. Foliar fertilization was done with the Bionat product. In relation to the specifics of each hybrid and the applied fertilization, the variation of some physiological and productivity parameters was recorded: plants height, Ph=179.80-203.60 ± 2.73 cm; inflorescence number, In=7.50-8.50 ± 0.12; flower number, Fn = 5.20-6.70 ± 0.17; fecundated flower, Ff = 4.60-6.20 ± 0.20; fruit number in bunches, Fnb = 4.60-6.20 ± 0.20; average bunch weight, Abw = 0.53-0.90 ± 0.05 kg; fruits number on plant, Fnp = 35.50-50.80 ± 2.12; average fruit weight, Afw = 0.12-0.16 ± 0.01 kg; average plant production, App = 4.17-7.33 ± 0.42 kg. Based on the coefficient of variation, high variability was recorded in the case of the App parameter (CV = 20.79140) and low variability in the case of the Ph parameter (CV = 3.95331). According to PCA, the distribution diagrams of variants were generated in relation to flowering parameters (PC1 explained 80.696% of variance, and PC2 explained 18.566% of variance) and in relation to fruiting parameters (PC1 explained 83.685% of variance, and PC2 explained 15.523% of variance).

Key words: *fecundated flowers, inflorescence number, multicriteria analysis, Pearson's correlation, prediction models.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) represent one of the most important horticultural species in the whole world, both in the production sector and in the agri-food and service chain (Tagiakas et al., 2022; Wako and Muleta, 2023). Tomatoes are cultivated plants, in the category of vegetables, cultivated both in protected spaces (greenhouses and solariums) and in the open field, with a major role in human nutrition (Bihon et al., 2022).

Tomatoes are characterized by a wide range of genotypes, among which F1 hybrids (commercial hybrids) predominate for crop, as a result of the advantages they offer (Hoza et al., 2022; Tagiakas et al., 2022). However, local germplasm (traditional plant populations) is also of interest as a result of some qualitative advantages of the fruits and some traditional cultivation systems, with socio-economic and ecological values. Tagiakas et al. (2022) compared several local tomato breeds (Greek) with commercial hybrids, and reported comparable results of the productivity indices and especially of the quality indices with the values recorded in the commercial hybrids. Although there are many cultivated genotypes, improving the yield and quality of the fruits is a permanent concern (Bihon et al., 2022).

Tomatoes have high specific nutritional requirements, and in order to optimize fertilization, different studies were done with mineral and organic mineral and biostimulant resources, with soil application, through fertigation, or foliar. Suchithara et al. (2022) reported favorable results for the use of microalgae in tomatoes, with variations in efficiency in relation to the application method. The authors of the study communicated the increase in the content of mineral elements in the fruits, and highlighted the option of fertilization with micoalge associated with manure.

The effects of mineral fertilization were analyzed in relation to the advantages on crops and yields in food production, and the side effects on the environment (Chaudhary et al., 2022). Vermicompost has been tested as an alternative to chemical fertilizers for adding nutrition to tomatoes (Qasim et al., 2023). Mixed fertilization, vercompost and mineral fertilizers, facilitated the reduction of the dose of chemical fertilizers. The authors of the study recorded better values for biometric parameters and physiological indices in plants, also for productivity elements, and fruit quality indices. The physical and chemical properties of the soil also registered a significant improvement, according to the authors.

Tomato production, fruit quality and tomato cultivation performance were studied in relation to the use of vermicompost in the plant culture system (Wako and Muleta, 2023). In the conditions of the study, the authors found variable profitability, often reduced in tomato crop as a result of limitations given by fertilizer resources, the level of training of growers and access to inputs. Analyzing the influence of vermicompost, the authors of the study highlighted positive aspects on the properties of the soil and in tomato crop, in terms of yield, fruit quality, with benefits for sustainable agriculture.

Biofertilizers find more and more use in horticultural practices, as a result of supporting crop productivity and a more environmentally friendly effect (Ammar et al., 2023). The use of biofertilizers is increasingly promoted within innovative, environmentally friendly technologies, increasingly promoted in the context of sustainable agriculture (Kumar et al., 2021).

In relation to tomato irrigation, as a component of culture technologies, the watering regime was studied to optimize the volume of water and the way the plants grow (Ahmad et al., 2023). Based on the data recorded regarding plant parameters, production and certain quality indices, the authors of the study identified the growth system and the watering regime in the appropriate variants for the tomato culture, in the specific study conditions.

Elements of productivity, yield and quality in tomatoes were studied in relation to the technique and watering regime, in order to optimize the use of water in arid and semi-arid areas (Mukherjee et al., 2023). The productivity of tomato crops was analyzed in relation to different categories of factors in order to optimize the production process and the profitability of farmers (Asfaw, 2021). The author used different sources of data collected from growers through appropriate sampling and statistical processing techniques, and the results provided information on the categories of factors and how they affected the productivity of the tomato crop. The productivity of the tomato crop was also analyzed in relation to the category of small farmers in relation to culture and marketing practices, in order to identify the constraints that affect farmers and formulate solutions (Nyalugwe et al., 2022). The study was based on the collection of data and information by questioning a significant number of respondents (farmers and input suppliers). The study provided results that led to the identification of some main categories of constraints that affected the productivity of the tomato crop under the study conditions, and facilitated the formulation of support solutions.

Productivity, fruit quality and profitability in tomatoes was studied in relation to fertilizer resources and crop variants with mulching and without mulching (Velza et al., 2023). The authors of the study recorded the variation of plant biometrics, the number of fruits and the quality of the fruits and the yield in relation to the type of fertilizer and culture technology.

The appropriate sources and elements were analyzed to optimize the tomato production process, especially for small farmers, in relation to tomato processors for the purpose of technical efficiency in the production chain, with efficiency for both components, producers - processors (Čechura et al., 2021).

In order to optimize tomato cultivation technologies, reduce the impact on the environment and sustainable production of food resources, comparative studies were made between tomato cultivation in the greenhouse and in the open field (Maureira et al., 2022). The authors of the study used simulation procedures and identified the impact of the two culture variants (greenhouse, open field) on some categories of inputs and environmental elements, with recommendations for reducing energy consumption and the use of clean energy resources.

Tomato cultivation technology was studied based on elements specific to intelligent agriculture (IoT elements) and precision agriculture, in order to monitor the water regime and optimize the watering regime of plants (Singh et al., 2023). The profitability of tomato cultivation was studied in relation to the

efficiency of the use of some categories of resources (Ajibare et al., 2022). Based on sampling techniques in stages, on representative categories of tomato growers, the study highlighted a series of factors that contribute to the profitability of tomato cultivation, in relation to the conditions of the study area. The authors of the study formulated practical recommendations for capitalizing on resources and increasing the level of profitability, under the specific conditions of the sampled growers.
A recent study analyzed productivity in tomato cultivation, in relation to different practices based on energy efficiency (Jerca and Smedescu, 2023). The authors of the study analyzed the evolution of greenhouse tomato production in Europe, the dynamics of surfaces and productivity.
To a significant extent, tomatoes are exploited through industrial processing, and in this sense some studies have analyzed the optimization of water and energy flows in the processing of tomatoes to optimize resources and reduce the impact on the environment (Eslami et al., 2023).
The present study analyzed the variation of some biometric parameters, physiological indices and productivity elements in four tomato hybrids (F1 hybrids) under the influence of treatments with the Bionat fertilizer product.

MATERIALS AND METHODS

The study took place in the area of Jimbolia, Timis County, Romania. The experiment was located in an unheated greenhouse, belonging to a family vegetable association (Figure 1).

Figure 1. Overview of the solariums where the tomato hybrid experiment was carried out

Four tomato hybrids were cultivated: Beldine F1; Sandolin F1; Ciciu F1; Izmir F1.
The tomato seedlings were 60 days old when planted. The culture was established in the solar soil, in rows in strips (40+80+80+80+40), with a density of 28,000 plants ha^{-1}.
The crop technology was specific to tomato culture, in unheated solariums. Within each hybrid, a control variant and a variant treated with the Bionat product were considered: control variants: Beldine F1 – V1; Sandolin F1 – V3; Ciciu F1 – V5; Izmir F1 – V7; variants treated with Bionat: Beldine F1 + Bionat – V2; Sandoline F1 + Bionat – V3; Ciciu F1 + Bionat – V5; Izmir F1 + Bionat – V7. The Bionat product was applied in four treatments, concentration 0.5%, at intervals of 14 days between treatments; the first treatment was applied after planting the seedlings.
In order to evaluate the influence of Bionat treatments, and the response of each hybrid, certain plant parameters were determined within each variant.
In relation to the specifics of tomato plants, the following parameters were determined: plant height (Ph, cm); inflorescence number (In, no.); flowers number in inflorescence (Fn, no.); fecundated flowers (Ff, no.); number of fruits in bunches (Fnb, no.); average bunch weight (Abw, kg b^{-1}, b - bunch); fruits number on plant (Fnp, no.); average fruit weight (Afw, kg f^{-1}, f -

fruit); average plant production (App, kg plt^{-1}, plt – plant).
The recorded experimental data were processed and analyzed mathematically and statistically appropriately (Hammer et al., 2001; Statistica, 2020; JASP, 2022).

RESULTS AND DISCUSSIONS

The determinations made during the vegetation period for tomato hybrids in relation to the applied treatments, led to the results presented in Table 1. The height of the plants varied between Ph = 179.80-203.60 ± 2.73 cm. The number of inflorescences was between In = 7.50-8.50 ± 0.12. The number of flowers in the inflorescence varied between Fn = 5.20-6.70 ± 0.17. The number of fertilized flowers (in the inflorescence) varied between Ff = 4.60- 6.20 ± 0.20. The number of fruits per bunch varied according to Fnb = 4.60-6.20 ± 0.20. It was found that there was no loss of fruits, the number of fruits at harvest per bunch was the same as the number of fecundated flowers. The average weight of the bunch varied between Abw = 0.53-0.90 ± 0.05 kg b^{-1}. The number of fruits per plant varied between Fnp = 35.50-50.80 ± 2.12. The average weight of a fruit was between Afw = 0.12 = 0.16 ± 0.01 kg f^{-1}. The average production per plant varied between App = 4.17-7.33 ± 0.42 kg plt^{-1}. The experimental data recorded showed heterogeneity and statistical reliability, according to ANOVA Test (Alpha = 0.001) (Table 2).

Table 1. The values of the parameters studied in tomatoes

Experimental Variants	Parameters determined in tomato plants								
	Ph	In	Fn	Ff	Fnb	Abw	Fnp	Afw	App
	(cm)	(no)	(no)	(no)	(no)	(kg b^{-1})	(no)	(kg f^{-1})	(kg plt^{-1})
V1	187.70	7.80	5.50	4.60	4.60	0.541	35.5	0.118	4.170
V2	196.40	8.40	5.90	5.50	5.50	0.705	46.3	0.128	5.925
V3	179.80	7.50	5.40	4.80	4.80	0.712	35.7	0.148	5.290
V4	198.30	8.10	6.20	5.70	5.70	0.900	46.5	0.158	7.330
V5	199.70	7.70	5.90	5.30	5.30	0.673	40.5	0.127	5.139
V6	203.60	8.20	6.70	6.20	6.20	0.863	50.8	0.139	7.074
V7	195.40	7.90	5.20	4.60	4.60	0.532	36.6	0.116	4.237
V8	199.80	8.50	5.90	5.50	5.50	0.709	46.9	0.129	6.057

Table 2. ANOVA Test

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	256312.8	8	32039.1	2943.579	5.24E-78	3.833807
Within Groups	685.7175	63	10.88441			
Total	256998.6	71				

Based on the correlation analysis, the values from Table 3 resulted. The height of the plants (Ph) and the number of inflorescences (In) showed lower correlations in intensity with the other determined parameters, as the productivity elements of the tomato plants. Very strong and strong correlations, statistically assured, were registered at the level of the parameters Fn, Ff, Fnb, Abw, Fnp.
Starting from the identified correlations, regression analysis was used to find out the variation of the Fnp (number of fruits per plant) and App (average fruit production per plant) parameters, as the main productivity parameters. The variation of the considered parameters (Fnp, App) in relation to the number of inflorescences (In) and the number of fecundated flowers (Ff) was described by the general equation (1), with the presentation of the values of the coefficients of the equation in Table 4. The graphic distribution of the values of Fnp and App in relation to In and Ff is presented in Figures 2 and 3.

$$Y = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: Y - Fnp, or App (presented in Table 4);
x - inflorescence number (In);
y - fecunded flowers (Ff);
a, b, c, d, e, f - coefficients of the equation (1) (Table 4);

Table 3. The table of correlations between parameters studied in tomatoes

	Ph	In	Fn	Ff	Fnb	Abw	Fnp	Afw	App
Ph									
In	0.671								
Fn	0.704	0.512							
Ff	0.739*	0.621	0.969***						
Fnb	0.739*	0.621	0.969***	.999***					
Abw	0.408	0.342	0.837**	0.864**	0.864**				
Fnp	0.783*	0.805*	0.901**	0.964***	0.964***	0.777*			
Afw	-0.059	-0.036	0.468	0.494	0.494	0.862**	0.376		
App	0.527	0.536	0.866**	0.916**	0.916**	0.976***	0.881**	0.766*	

Table 4. Statistical values related to equation (1)

Coefficient	Y = Fnp (according to Eq. 1)				Y = App (according to Eq 1)			
	Coefficient values	R^2	F	RMSE	Coefficient values	R^2	F	RMSE
a	-1.6971501	0.999	408.021	0.1758	-8.9477960	0.935	5.7636	0.2801
b	-0.4133510				-3.6046885			
c	23.0813388				76.0047572			
d	-2.8035737				-61.9891430			
e	1.8897685				12.7655628			
f	-87.1202972				-140.5404017			

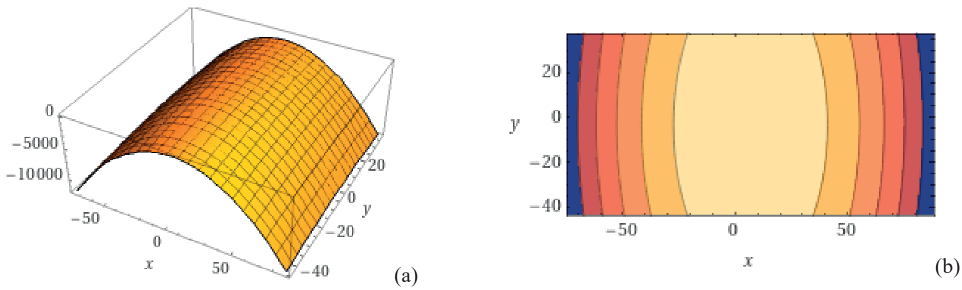

Figure 2. The graphic distribution of the parameter Fnp in relation to In and Ff in tomatoes

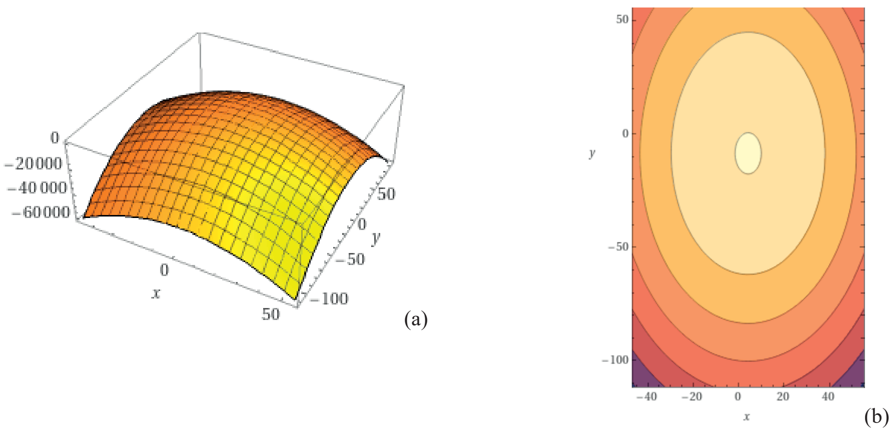

Figure 3. The graphic distribution of the App parameter in relation to In and Ff in tomatoes

PCA analysis was used to find out the distribution of variants in relation to parameters associated with flowers and fruits. In relation to flower parameters (In, Fn, Ff), the diagram in

Figure 4 resulted, in which PC1 explained 80.696% of variance, and PC2 explained 18.566% of variance. In relation to fruit parameters, the diagram in Figure 5 resulted, and PC1 explained 83.685% of variance, and PC2 explained 15.523% of variance.

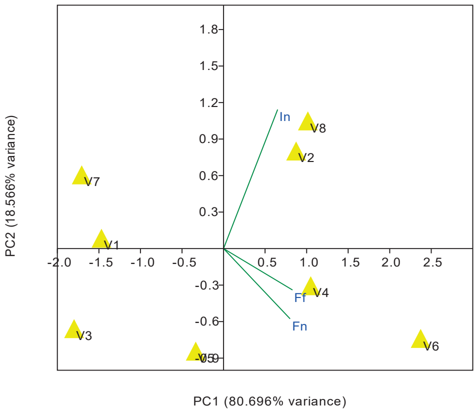

Figure 4. PCA diagram in relation to representative parameters for tomato flowers

The increase generated by Bionat treatments was analyzed for each analyzed parameter. For this, the significance of the differences between the mean of the control variants (Ct; V1, V3, V5, and V7), and the values of the variants treated with Bionat (V2, V4, V6, and V8) was analyzed. The results obtained (One-sample test) are presented in Table 5.

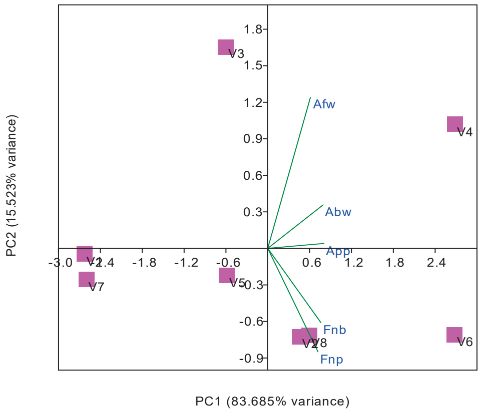

Figure 5. PCA diagram in relation to representative parameters for tomato fruits

The differences between the mean of the control variant (Ct) and the sample mean showed statistical reliability in the case of parameters Ph, In, Ff, Fnb, Fnp and App ($p < 0.05$). In the case of the Fn, Abw and Afw parameters, the differences did not show statistical certainty.

Table 5. The significance of the differences for the parameters studied in tomatoes

Statistical parameters	Parameters determined in tomato plants								
	Ph	In	Fn	Ff	Fnb	Abw	Fnp	Afw	App
Given mean:	190.65	7.725	5.50	4.825	4.825	0.614	37.075	0.127	4.709
Sample mean:	197.75	8.185	6.04	5.545	5.545	0.7582	45.515	0.1362	6.219
95% conf. interval:	(191.83 203.67)	(7.8102 8.5598)	(5.4875 6.5925)	(4.9318 6.1582)	(4.9318 6.1582)	(0.6098 0.9066)	(39.224 51.806)	(0.11993 0.15247)	(4.9229 7.5151)
Difference:	7.10	0.46	0.54	0.72	0.72	0.1442	8.440	0.0092	1.510
95% conf. interval:	(1.1789 13.021)	(0.08518 0.83482)	(-0.012506 1.0925)	(0.10682 1.3332)	(0.10682 1.3332)	(-0.0041968 0.2926)	(2.1494 14.731)	(-0.0070701 0.02547)	(0.21394 2.8061)
t :	3.3293	3.4074	2.7136	3.2601	3.2601	2.6979	3.7251	1.57	3.2348
p (same mean):	0.029126	0.027092	0.053338	0.031077	0.031077	0.054211	0.020384	0.19151	0.031832
Significance	*	*	ns	*	*	ns	*	ns	*

The height of the plants (Ph) in the case of the treated variants showed significant differences compared to the control variant (Ct), average values. The height of the plants was positively correlated with the parameters Ff, Fnb and Fnp (*, $p<0.05$) (Table 3). Through the positive variation of the height of the plants, with a positive impact on the three productivity parameters, the treatment with Bionat was justified. The number of inflorescences (In) showed significant differences in the treated varieties, compared to the average of the control varieties, and showed a positive correlation with Fnp ($r = 0.805$*).

In the case of the number of flowers (Fn), the treatment with Bionat did not generate statistically significant differences (Table 4). However, the Fn parameter presented positive correlations, very strong with Ff, Fnb and strong with Abw, Fnp and App. This may suggest the physiological balance of tomato plants.

If we still analyze the number of fertilized flowers (Ff), it was found that in the case of the treated variants, the recorded results showed differences compared to the average Ct, under statistically reliable conditions ($p < 0.05$). On the one hand, this shows the bioactive effect of the Bionat product, and on the other hand, it justifies the treatment in the production process.

In the case of the Ff parameter (fecundated flowers), treatments with Bionat generated statistically safe increases, the differences between the mean of the treated variants and the control variants showed statistical reliability ($p < 0.05$). The Ff parameter presented very strong, positive correlations with Fnb and Fnp, and strong, positive correlations with Abw and App. A similar response was recorded in the case of the Fnb parameter.

In the case of the Abw parameter, the differences generated by the treatment with Bionat, in relation to the average of the control variants, did not show statistical certainty. However, Abw presented positive correlations with other determined parameters, under conditions of statistical safety. Similar results were recorded in the case of the Afw parameter.

The values of the Fnp parameter, in the case of the variants treated with Bionat, showed differences compared to the average of the control variants, under statistical safety conditions. The Fnp parameter presented positive correlations, with the other parameters, under statistical safety conditions.

In the case of the App parameter in the treated variants, differences were recorded compared to the control variants, under statistical safety conditions, table 4. The App parameter showed significant correlations with the other studied parameters, except for Ph and In.

Increased yield and better quality indices for vegetable products have been reported in different horticultural species in relation to differentiated fertilization systems (Dobrei et al., 2009; Ofoe et al., 2024). Yu et al. (2023) communicated models for managing the watering and fertilization rate for productivity efficiency in greenhouse tomatoes. The positive variation of the photosynthetic indices and the quality indices, under statistical safety conditions, was recorded in relation to the variable rate of tomato fertilization in greenhouse conditions (Ofoe et al., 2024). Organic fertilization generated favorable results for tomatoes, in terms of the yield and the content of mineral elements in the fruits (Adekiya et al., 2022). Through the recorded results, the present study shows, on the one hand, the differentiated response of the cultivated tomato hybrids, and on the other hand, highlights the favorable bioactive effect of the Bionat fertilizer used, and thus contributes a set of information to the scientific literature in the specific field of the tomato crop.

CONCLUSIONS

The four F1 tomato hybrids have differently valorised the treatments with the applied Bionat fertilizer. The highest value for App was recorded in the Sandoline F1 hybrid, under the influence of Bionat treatment (V4; App = 7.330 kg plt^{-1}). In second place was the hybrid Ciciu F1, under the influence of Bionat treatment (V6; App = 7.074 kg plt^{-1}).

Treatments with Bionat generated increases in the studied parameters, compared to the control variant (average of the untreated variants, V1, V3, V5, V7), under statistical safety conditions ($p < 0.05$) for the parameters Ph, In, Ff, Fnb, Fnp and App. Positive differences were also recorded in the case of the other parameters (Fn, Abw, Afw), but without statistical certainty. A favorable effect of the treatment with Bionat was registered in the case of the parameter Ff (fecundated flowers), even if the parameter Fn (total number of flowers) did not register a positive variation, in statistical safety conditions, under the influence of the applied fertilizer. The results show the importance of the treatment for the growth of fruits number on the plant.

The statistical analysis used, generated results in the form of equations and graphic models, which described the variation of the main parameters of tomato productivity, under the study conditions.

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ASSESSMENT THE EFFECT OF GENOTYPE X MYCORRHIZA INTERACTION ON SOME FRUIT QUALITY TRAITS IN TOMATO

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Abstract

In the context of current concerns regarding sustainable tomato production, the use of arbuscular mycorrhizal fungi (AMF) can represent an important way in order to reduce the use of chemical fertilizers and pesticides and their negative environmental impact. The aim of this study was to assess the qualitative traits of fruits (firmness, titratable acidity, total soluble solids, maturity index, and flavour index) in six tomato genotypes under the effect of mycorrization with Glomus sp. The biological material was composed by five hybrids and one tomato variety developed at ULS Timisoara. The study was carried out using a split-plot design with mycorrhizal treatment as the main factor. The effect of AMF on different quality traits was influenced by the genotype, to a greater extent for fruit firmness and acidity, and associated with an increase of sugar content and firmness of fruits. The obtained results highlighted that the use of AMF in tomato can lead to an improvement in fruit quality, considering that a selection of appropriate genotypes is also necessary.

Key words: *Solanum lycopersicum, arbuscular mycorrhizal fungi, fruit quality.*

INTRODUCTION

Tomato, is one of the highest-value vegetable with an extensive worldwide distribution. Quality is considered an important factor for ensuring higher value of tomato fruits, being influenced by the interaction of genotype with several ecological and technological factors. In tomato production system, the arbuscular mycorrhizal fungi (AMF) can be an effective alternative n order to reduce the input of chemicals and their environmental impact (Toju et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs and are the most widespread symbioses with the roots of the majority important crop species (Smith & Read, 2008), and enhance their mineral nutrition by improving the absorption of several nutrients, in exchange for the use of carbon compounds resulted from the photosynthetic process (Bucher et al., 2014; Keymer et al., 2017; Nedorost & Pokluda, 2012).

AMF can modify root hydraulic properties (Bárzana et al., 2012) that will increase the water supply in shoots and tolerance to water stress (Bowles et al., 2016; Chitarra et al., 2016), also the AMF plants can express a higher net photosynthetic rates (Birhane et al., 2012; Bowles et al., 2016; Huang et al., 2011). Inoculation with AMF decreased stem elongation in plants under low light conditions, thus counteracting the plant's reaction to shading (Sahaet al., 2022). The mechanisms that produce benefits of AMF are depend on the stress characteristics, and are mostly regulated by phytohormones (Pozo et al., 2015). The beneficial effects of AMF on plant growth that were frequently observed has stimulated the development of several biostimulant products for agricultural use (Lee Diaz et al., 2021).

Tomato is one of the most important crops which are known to have several benefits from symbiotic relationships with mycorrhizae. The use of AMF provide benefits to the development of tomato plants, improved their health and might be of particular interest for ecological tomato production (Jamiołkowska et al., 2020). The symbiosis between roots and AMF in tomato provides nutrients and water to the host plant, thus stimulating plant growth and increasing yield and fruit quality (González-González et al., 2020; Paskovic et al., 2021; Saia et al., 2019). The colonization of tomato roots with AMF increase the resistance of plants to biotic (Aseel et al., 2019; Song et al., 2015) and abiotic stresses (Liang et al., 2022; Volpe et al., 2018). Mycorrhizal colonization in tomato produce a defense mechanism that induced effective resistance against fungal and bacterial, virulent and avirulent pathogens, insects and necrotrophs (Fujita et al., 2022; Nguvo & Gao, 2019).

Under the inoculation with AMF, the tomato plants were more vigorous having a developed root system (Felföldi et al., 2022). Mycorrhizal inoculated tomato plants flowered earlier (Ortas et al., 2013), produced larger inflorescences and a higher number of flowers (Conversa et al., 2013; Urias-Garcia et al., 2022) and has uniform fruits (Dasgan et al., 2008). As a consequence of early flowering, the AMF treatment increase the first harvest yield and reduce the harvesting time (Candido et al., 2013; Salvioli et al., 2012). The benefits of AMF on tomato growth can be due to the association between the mycorrhizal plants colonization and the quantity and quality of pollen. (Dey & Ghosh, 2022).

The inoculated tomato plants exhibited an increase of sweetness and a reduction of fruit acidity (Chafai et al., 2023; Ullah et al., 2023), and improved quality of nutrients by increasing the citric acid, antioxidants, carotenoids, amino acids (Bona et al., 2017; Di Fossalunga et al., 2012; Miranda et al., 2015). The beneficial effect of AMF symbiosis on the accumulation of bioactive compounds in fruits is dependent on the AMF species, host plant and growing conditions (Horvath et al., 2020).

The previous findings highlighted that the utilization of AMF can be an effective way to obtain yield with high-quality fruits and to reduce the fertilizer doses and their environmental impact. In this context, the aim of this study was to assess the qualitative traits of fruits (firmness, titratable acidity, total soluble solids, maturity index, and flavor index) in six tomato genotypes under the effect of mycorrization with *Glomus* sp.

MATERIALS AND METHODS

The biological material was composed by four F_1 hybrids ('Banato', 'Miruna', 'Sorada', 'USAB29') and one tomato variety ('Tomtim') developed at ULS Timisoara, and 'Ghittia' Romanian variety.

The study was carried out using a split-plot design in three replications, with mycorrhizal treatment (no AMF; with AMF) as the main factor and the genotype as second factor. The 45 old days seedlings were transplanted into the field on May 10, 2021, using a density of 3.33 plants/m^2 (0.7-0.8 m between the rows/0.4 m on the rows).

Inoculation with *Glomus* sp., was done during the transplanting of seedlings into the field, by placing 4.5 g of inoculums consisting of 1 x 10^7 CFU/g (50% *Glomus intraradices* + 50% *Glomus mosseae*), in the planting holes. As a source of mycorrhiza the commercial product Aegis Pastiglia (ITALPOLLINA S.p.A., Italy) was used.

The soil was mulched with black plastic film and the irrigation was done by drip. Cultural practices for tomato field crop were applied. The average monthly temperatures ranged between 21.9ºC in May to 32.5ºC in July, while the average monthly rainfall ranged from 28.4 mm in May to 47.5 mm in July.

Ten representative mature fruits for each mycorrhizal treatment and genotype were randomly selected for analyses. The total soluble solids (TSS) was carried out using a digital refractometer (DR 201-95) and the results were expressed in ºBrix. The fruit firmness was measured using a penetrometer (PCE – PTR 200) and the results were expressed in kg/cm^2.

The titratable acidity (TA) was determined through a titration procedure using a NaOH (0.1 N) solution, and calculated using the formula (Saad et al., 2014):

TA (citric acid %) = (v x N x 100 x 0.064)/m, where: v-volume (mL) of NaOH solution; N - 0.1; 0.0064 - conversion factor for citric acid; m-sample mass (g).

The maturity index (MI) and flavor index were calculated with the following formulas (Hernandez Suarez et al., 2008):

MI = TSS/TA; FI = TA + [(TSS/20) x TA]. The means were compared using ANOVA and Least Significant Difference test (Ciulca, 2006). The genotypes were clustered using the UPGMA algorithm (Neighbor program) of the Phylip package. The basic principles of the biplot technique were used to display in a graph the performance of each genotype for the analyzed traits.

RESULTS AND DISCUSSIONS

Regarding the main effect of mycorrhization (Table 1), the fruits firmness showed amplitude of 0.49 kg/cm^2 with values ranging from 2.52 to 3.01 kg/cm^2. Mycorrhization had a slight positive effect causing a significant increase of 19.44%. Considering the cumulative effect of the genotype, the average values of fruit firmness ranged from 1.99 kg/cm^2 in the Banato hybrid to 3.51 kg/cm^2 in the case of the Ghittia variety. The plants of Ghittia variety and Sorada hybrid have used this year's growing conditions at a higher level, achieving a superior fruit firmness compared to the other genotypes. The fruit firmness of USAB29 hybrid was significantly higher than Tomtim, Miruna and Banato genotypes.

Table 1. The effect of mycorrhization and genotype on tomato fruits firmness (kg/cm^2)

Genotype	Mycorrization		Genotype	
	AMF -	**AMF +**	Mean	CV
Banato	1.82 d	2.16 d	1.99±0.09 D	18.39
Ghittia	3.25 a	3.78 a	3.51±0.16 A	18.85
Miruna	1.83 d	2.33 d	2.08±0.12 D	24.02
Sorada	3.00 ab	3.82 a	3.41±0.15 A	18.19
Tomtim	2.28 c	2.66 c	2.47±0.08 C	14.23
USAB29	2.96 b	3.29 b	3.13±0.12 B	15.68
AMF Mean	2.52±0.09 Y	3.01±0.11 X	2.77±0.08	
CV	28.74	26.33	28.70	

AMF- (non-mycorrhizal); AMF+ (mycorrhizal); Data represents mean ±SE
Genotype LSD 5%=0.21; AMF LSD 5%=0.19; Genotype x AMF LSD 5%=0.26
Different letters (a-d) in the columns indicate significant differences ($p<0.05$) between genotypes.
Capital letters were used for AMF means (X, Y) and genotype means (A-D) comparisons.

The mycorrhization caused a significant increase in fruit firmness at the plants of all genotypes, being associated with increases between 11.15% at USAB29 and 27.33% in Sorada hybrid. The non-mycorrhizal plants (AMF-) of the genotypes achieved fruits firmness values from 1.82 kg/cm^2 for Banato hybrid up to 3.25 kg/cm^2 at Ghittia variety, while in the mycorrhizal plants (AMF+) the amplitude was higher ranging from 2.16 kg/cm^2 in Banato to 3.82 kg/cm^2 in Sorada hybrid. Independent of mycorrhization, it was observed that Ghittia variety and Sorada hybrid have recorded significantly higher values compared to the other genotypes.

Under the effect of AMF, at the level of the whole experience the TSS of the fruits recorded amplitude of 0.36 with limits from 5.98 to 6.34 ^{0}Brix (Table 2). Thus, it was observed that the use of AMF had a positive effect associated with a significant increase in the amount of TSS by 6.02%. Given the unilateral effect of the genotype, it was observed that the TSS showed a variation of 0.46 ^{0}Brix with values between 5.88 in USAB29 and 6.34 ^{0}Brix in Tomtim variety. As such, Ghittia and Tomim varieties have achieved significantly lower values compared to the rest of the genotypes. The four hybrids registered significantly equal values of TSS.

Table 2. The effect of mycorrhization and genotype on TSS (^{0}Brix) of tomato fruits

Genotype	Mycorrization		Genotype	
	AMF -	**AMF +**	Mean	CV
Banato	6.07 ab	6.32 ab	6.19±0.05 A	3.86
Ghittia	5.86 bc	6.16 bc	6.01±0.08 B	5.73
Miruna	6.11 a	6.49 a	6.30±0.09 A	6.01
Sorada	6.02 ab	6.45 a	6.24±0.07 A	4.71
Tomtim	5.65 c	6.10 c	5.88±0.08 B	5.64
USAB29	6.17 a	6.51 a	6.34±0.07 A	4.85
AMF Mean	5.98±0.04 Y	6.34±0.03 X	6.16±±0.03	
CV	5.26	4.58	5.70	

AMF- (non-mycorrhizal); AMF+ (mycorrhizal); Data represents mean ±SE
Genotype LSD 5%=0.16; AMF LSD 5%=0.1; Genotype x AMF LSD 5%=0.22
Different letters (a-c) in the columns indicate significant differences ($p<0.05$) between genotypes.
Capital letters were used for AMF means (X, Y) and genotype means (A-B) comparisons

Based on the genotype x mycorrization interaction, it was observed that in the AMF- plants the amount of soluble solids varied from 5.65 in Tomtim variety up to 6.17 ^{0}Brix for USAB29 hybrid, with amplitude of 0.52 ^{0}Brix. The USAB29 and Miruna hybrids achieved significant increases than Ghittia and Tomtim varieties.

Under the effect of AMF the variation of TSS was associated with amplitude from 6.1 in Tomtim variety up to 6.51 ^{0}Brix for USAB29 hybrid. In the AMF+ plants, the fruits of Miruna, Sorada and USAB29 hybrids were highlighted, which presented a significantly higher TSS compared to Ghittia and Tomtim varieties. Regarding the effect of mycorrization on the TSS in each genotype, it was found that generally AMF+ plants recorded significant relative increase from 4.12% in Banato to 7.96% in Tomtim.

Regarding the main effect of mycorrhization (Table 3), the fruits titratable acidity (TA) showed amplitude of 0.086 with values ranging from 0.46 to 0.546 citric acid %. Overall, the mycorrhization had an negative effect causing a significant relative decrease of 15.75%.

Given the cumulative effect of the genotype, average values of TA from 0.447 in Sorada to 0.593 citric acid % in Tomtim variety, were found. The fruits of Tomtim and Banato achieved a significantly higher acidity by 9.5-47.1% than the rest of the genotypes. The acidity of Miruna fruits was significantly lower to the other genotypes.

Table 3. The effect of mycorrhization and genotype on titratable acidity (citric acid %) of tomato fruits

Genotype	Mycorrization		Genotype	
	AMF -	**AMF +**	Mean	CV
Banato	0.602 ab	0.530 a	0.566±0.023 A	17.34
Ghittia	0.573 bc	0.450 b	0.512±0.024 B	19.80
Miruna	0.437 e	0.370 c	0.403±0.014 D	14.73
Sorada	0.487 d	0.407 b	0.447±0.019 C	18.75
Tomtim	0.623 a	0.563 a	0.593±0.026 A	18.76
USAB29	0.553 c	0.440 b	0.497±0.028 B	24.37
AMF Mean	0.546±0.014 X	0.460±0.015 Y	0.503±0.011	
CV	19.34	23.95	23.01	

AMF- (non-mycorrhizal); AMF+ (mycorrhizal); Data represents mean ±SE
Genotype LSD 5%=0.031; AMF LSD 5%=0.024; Genotype x AMF LSD 5%=0.042
Different letters (a-e) in the columns indicate significant differences ($p<0.05$) between genotypes.
Capital letters were used for AMF means (X, Y) and genotype means (A-D) comparisons.

The mycorrization significantly influenced the TA of fruits for all genotypes, being associated with decreases between 9.63% in Tomtim and 21.47% in Ghittia variety. The AMF- plants registered an TA of the fruits with values from 0.437 for Miruna to 0.623 citric acid % in Tomtim, while in the AMF+ plants the amplitude was between 0.370 and 0.563 citric acid %. In the absence of mycorrization the fruits of the Tomtim variety had a significantly higher TA to the other genotypes, while under the effect of mycorrization the fruits of Banato next to the Tomtim fruits, recorded a high TA. Independent of mycorrization, the fruits of Banato hybrid showed a significantly lower acidity.

Table 4. The effect of mycorrhization and genotype on maturity index of tomato fruits

Genotype	Mycorrization		Genotype	
	AMF -	**AMF +**	Mean	CV
Banato	10.40 cd	12.11 d	11.26±0.48 E	17.99
Ghittia	10.36 cd	14.14 c	12.25±0.68 D	23.57
Miruna	14.25 a	17.76 a	16.00±0.65 A	18.08
Sorada	12.65 b	16.51 ab	14.58±0.84 B	24.44
Tomtim	9.32 d	11.34 d	10.33±0.59 F	24.41
USAB29	11.45 bc	16.23 b	13.84±1.04 C	35.11
AMF Mean	11.40±0.34 Y	14.68±0.55 X	13.04±0.36	
CV	22.04	27.56	29.63	

AMF- (non-mycorrhizal); AMF+ (mycorrhizal); Data represents mean ±SE
Genotype LSD 5%=0.98; AMF LSD 5%=0.66; Genotype x AMF LSD 5%=1.34
Different letters (a-d) in the columns indicate significant differences ($p<0.05$) between genotypes.
Capital letters were used for AMF means (X, Y) and genotype means (A-E) comparisons.

Under the effect of AMF, at the level of the whole experience the maturity index (MI) of the fruits recorded amplitude with limits from 11.4 to 14.68 (Table 4). Thus, it was observed that the use of AMF had a positive effect associated with a significant increase of MI by 28.77%.

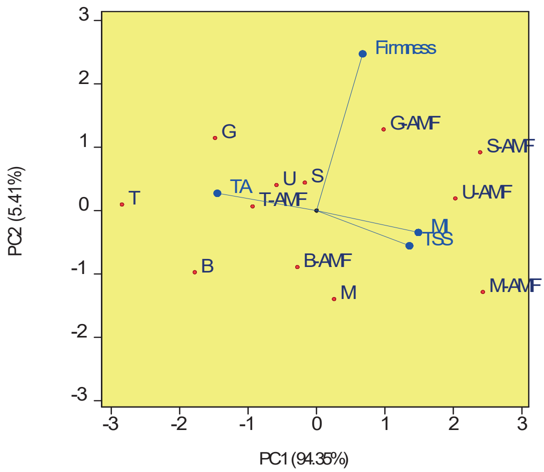
Given the main effect of the genotype, it was observed that the MI shoved a variation of 5.67 with values from 10.33 in Tomtim to 16 in Miruna hybrid. As such, the fruits of Miruna have achieved significantly higher value compared to the rest of the genotypes, being followed by those of Sorada and USAB29.

Based on the genotype x mycorrization interaction, it was observed that in the AMF- plants the MI varied from 9.32 in Tomtim up to 14.25 for Miruna. As such, the fruits of Miruna achieved significant increases of flavor and taste, while the fruits Sorada have been highlighted by a superior aroma against Banato, Ghittia and Tomtim fruits. Under the effect of mycorrization the variation of MI was associated with amplitude from 11.34 in Tomtim up to 17.76 for Miruna. Also, in the AMF+ plants, the fruits of Miruna and Sorada hybrids were highlighted, by a higher flavor and taste. Regarding the effect of mycorrization on the MI in each genotype, it was found that generally AMF+ plants recorded significant relative increase from 16.44% in Banato to 41.75% in USAB29 hybrid.

Table 5. The effect of mycorrhization and genotype on flavour index of tomato fruits

Genotype	Mycorrization		Genotype	
	AMF -	**AMF +**	Mean	CV
Banato	0.785 a	0.697 a	0.741±0.03 A	17.33
Ghittia	0.742 a	0.588 b	0.665±0.031 B	19.72
Miruna	0.570 b	0.490 c	0.530±0.017 C	13.93
Sorada	0.632 b	0.537 bc	0.585±0.024 C	17.60
Tomtim	0.799 a	0.735 a	0.767±0.033 A	18.32
USAB29	0.723 a	0.582 b	0.653±0.036 B	23.47
AMF Mean	0.709±0.018 X	0.605±0.019 Y	0.657±0.014	
CV	19.04	23.39	22.38	

AMF- (non-mycorrhizal); AMF+ (mycorrhizal); Data represents mean ±SE
Genotype LSD 5%=0.06; AMF LSD 5%=0.012; Genotype x AMF LSD 5%=0.078
Different letters (a-c) in the columns indicate significant differences (p<0.05) between genotypes.
Capital letters were used for AMF means (X, Y) and genotype means (A-C) comparisons.



B - Banato; G - Ghittia; M - Miruna; S - Sorada; T - Tomtim; U - USAB29

Figure 1. Biplot (PC 1, 2) for fruits quality traits of tomato genotypes

The mycorization significantly influenced the FI of fruits for most of the genotypes, being associated with decreases between 11.21% in Banato and 20.75% in Ghittia variety (Table 5). The FI of Tomtim variety fruits was not significantly affected by the mycorrization.

The AMF- plants registered an FI of the fruits with values from 0.57 for Miruna to 0.799 in Tomtim, while in the AMF+ plants the amplitude was between 0.49 and 0.735. In the absence of mycorrization the fruits of Miruna and Sorada hybrids have had a significantly lower flavour to the other genotypes, while under the effect of mycorrization the fruits of Banato next to the Tomtim fruits, recorded the highest flavour.

The PCA biplot from Figure 1 expresses 99.76% of the variability of the four fruit quality traits in the six tomato genotypes. Depending on the position of the genotypes towards the vectors of the different traits, it was observed that overall the AMF+ plants achieved higher MI and TSS, associated with low TA. The AMF+ plants of Sorada and USAB29 hybrids achieved fruits with the highest flavour and aroma according to MI and TSS, associated with lower TA and firmness above the mean, while the fruits of AMF+ Miruna plants shoved high TSS, MI, low TA and firmness. In the case of AMF+ plants for Tomtim variety the fruits recorded the high TA, low TSS, MI and average firmness. Under the mycorrization the fruits of Banato and Ghittia shoved similar TA, TSS and MI, but different firmness, higher in Ghittia, respectivelly. Regarding the AMF- plants, the fruits of Miruna hybrid achieved the higher taste and flavour and lower firmness.

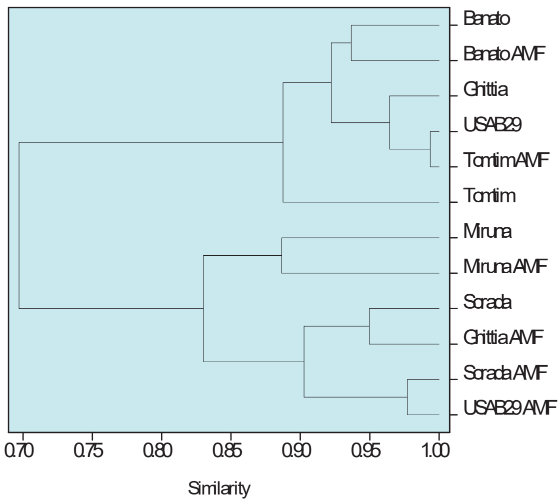


Figure 2. UPGMA clustering of tomato genotypes based on fruits quality traits

According to the hierarchical classification (Figure 2), it has been observed that at AMF- plants, the fruits highest similarity for quality traits was recorded for the genotypes: USAB29 and Ghittia (96.45%); USAB29 and Sorada (95.88%); Banato and Tomtim 93.67%). The highest diversity of fruits quality traits, was presented by the AMF- plants: Miruna and Tomtim (35.35%); Miruna and Ghittia (28%).

Considering the AMF+ plants, the highest similarity was observed between the fruits of the following genotypes: Sorada and USAB29 (97.71%); Banato and Tomtim (96.637%); Ghittia and Sorada 95.02%), while the genotypes Tomtim and Miruna (40.44%), Tomtim and Sorada (33.36%), genotypes showed a high fruits diversity. Thus, generally under the effect of mycorrization, an decrease of the similarity for the fruits quality traits between different genotypes was observed, which indicates a different reaction of them. The AMF- plants of Banato, Ghittia, Tomtim, USAB29 and AMF+ plants of Banato, Tomtim, are clustered togheter in a main group, showing a 90% similarity of the fruits quality traits. The fruits of these plants are characterised by high acidity, low soluble solids content and maturity index. The AMF+ plants of Miruna, Ghittia, Sorada, USAB29, and AMF- plants of Miruna, Sorada, constitute a second major group characterized by a similarity of 83%.

Regarding the significant negative impact of mycorrhization on titrable acidity of fruits Bona et al. (2017) reported similar results, while Regvar et al. (2003) found an association of low acidity with a slight decrease in electrical conductivity.

The beneficial effect of mycorrhiza symbiosis on the increase of total soluble solids as an indicator of dissolved, sucrose, and fructose, which reflects fruit sweetness, aligns with previous research (Chafai et al., 2023; Ullah et al., 2023). Our finding are in accordance with that of previous studies (Horvath et al., 2020; Huang et al., 2013; Subramanian et al., 2006) who found higher content of sugar in fruits of mycorrhizal plants under different stresses.

The positive effect of mycorrhization on fruit firmness and sugar content previous reported by Felfoldi et al. (2022), has been confirmed in our study. The increased fruit yield and quality of mycorrhizal tomato plants was also reported by previous studies (Di Fossalunga et al., 2012; Giovanetti et al., 2012; Miranda et al., 2015).

CONCLUSIONS

The mycorrhiza treatment expressed the highest effect on the variability of sugar content and a lower effect on the fruits firmness, being associated with an increase of firmness, sugar content and maturity index, and a decrease of acidity and flavour index, respectively.

The fruits firmness shoved the highest variability between genotypes, while the variation of sugar content was lesser influenced by the genotype.

Under the effect of genotype x mycorrhiza interaction, the acidity of fruit recorded the highest variability, in opposite with the low influence on fruit firmness.

In the case of fruits from Tomtim hybrid and Ghittia variety the highest effect of mycorrhiza treatment were observed, while the influence on the fruits from Banato hybrid was lower.

The mycorrhizal plants of Sorada and USAB29 hybrids achieved fruits with the highest sugar content, flavour and aroma associated with lower acidity and a good firmness, while the quality of Miruna fruits were associated with a low firmness.

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ANALYSES OF MORPHOLOGICAL DYNAMICS INTO THE VEGETATIVE PHASE OF WHITE CABBAGE (*BRASSICA. OLERACEA* VAR. *CAPITATA* F. *ALBA*) ACROSS DIVERSE PLANTING SCHEDULES

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Abstract

Cabbage (Brassica oleracea var. capitata f. alba) stands as an economically significant vegetable crop cultivated on a global scale. The life cycle encompasses four distinct vegetative stages: seedling, rosette, folding, and heading. Rosette leaves, contribute substantially to the energy allocation necessary for the development of the characteristic leafy head. This study delves, firstly, into a detailed morphological analysis to investigate the early developmental stages of cabbage seedlings, unravelling the intricate dynamics of their overall morphology. Secondly, an exploration was carried out into the quantities of assimilatory pigments within the cabbage seedlings which provides valuable aspects of early cabbage development. The third study delves into the influence of planting dates and densities on the morphological characteristics of cabbage plants during the vegetative phase. Through analyses of plant morphology, the study discerns nuanced developmental responses influenced by varied planting schedules. This research aims to contribute not only to the scientific understanding of white cabbage cultivation but also to practical implications for optimizing planting strategies.

***Key words**: seedlings, carotenoid pigments, planting strategies, Brassicaceae, heading stage.*

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata* f. *alba*) is a vegetable plant belonging to the *Brassicaceae* family, recognized as the largest plant family. As a cool-season vegetable characterized by short roots, cabbage is primarily cultivated for its substantial leafy head. Its cultivation traces its origins to Western Europe, with historical records attesting to its initial introduction and cultivation in the region (Singh et al., 2006).
The cultivation of this plant primarily focuses on its sizeable foliage. The morphological attributes of various cabbage cultivars, including head size, shape, colour, and leaf texture, exhibit considerable diversity (Nieuwhof, 1969).
Cabbage leaves are recognized for their considerable nutritional value, containing antioxidant phytochemicals (Singh et al., 2006, Sharma et al., 2018), a spectrum of macro- and micronutrients (Turan et al., 2014), and notable levels of vitamins C, E, and K (Ibukunoluwa Moyin-Jesu, 2015). Cabbage holds a prominent position among vegetables, serving as a crucial component in maintaining a healthy diet. A sample of 100 g leaves contains 93 mL water, 15 g protein, 0.2 g fat, 4 g carbohydrates, 4 g calcium, and 0.5 g iron (Moamogwe, 1995). Cabbage is rich in essential micronutrients pivotal for human health and plant functions, including copper, zinc, and iron (Nkosi and Msimango, 2022).
White headed cabbage stands out and attracts consumer preference due to its superior nutritional profile, providing significant health benefits for human consumption. Rich in vitamins, fibres, polyphenols, and flavonoids, it represents a valuable source of nutrients (Nawaz et al., 2018).
Initially, its leaves exhibit a rosette-like configuration and subsequently develop compound heads composed initially of 7-15 leaves that fold inward (Chura et al., 2021).
Plants quality relies on plant genotypes, environmental conditions, and agro-management practices (Gruda, 2019). While

genetic material is acknowledged for its impact on quality, seedlings are primarily focused on increasing yield, optimizing harvest timing, and addressing plant diseases and abiotic stress. Early utilization of high-quality planting material in cabbage crops substantially enhances overall success, efficiency, performance, hastens time to harvest, and improves profitability, regardless of the cultivation environment.

Leafy vegetables, particularly cabbage, demand high levels of nitrogen, phosphorus, and potassium, especially nitrogen fertilizer (Pradhan et al., 2007). However, excessive and indiscriminate use of chemical fertilizers can raise planting costs and pose environmental risks. Thus, enhancing fertilization practices and fertilizer efficiency is critical for supporting sustainable development in agriculture.

The proper selection of planting date and plant spacing significantly influences cabbage production practices by affecting yield-contributing factors and overall harvest. These decisions also play a primary role in influencing the quality attributes of cabbage heads.

The utilization of appropriate high-yielding cultivars and optimal spacing techniques could aid farmers in attaining increased yields per unit area, along with enhanced nutrient assimilation and solar energy capture efficiency. Variations in both morphological characteristics and phenological responses among cultivars lead to differential reactions to plant density (Prasad et al., 2010). Furthermore, diverse cultivars exhibit discrepancies in growth, yield, and quality attributes, which are contingent upon varying environmental conditions (Thapa et al., 2012).

Optimal cabbage plant population recommendations commonly range from 20.000 to 70.000 plants ha^{-1} (Ghanti et al., 1982; Tenday and Kuzyk, 2001; Kumar and Rawat, 2002). However, these findings report inconclusive results.

The response of cabbage yield and quality to plant density is influenced by various factors, including plant genotype, climate conditions, soil, water regime, nutrient status, market requirements, and others (Parmar et al., 1999; Tiwari et al., 2003).

Increasing plant population in cabbage has the potential to enhance yield and profit. While high plant density in cabbage may reduce head weight and size (Csizinszky and Schuster, 1985), a greater number of heads per unit area has been shown to increase total yield (Stepanović et al., 2000). Maximizing benefits in cultivation systems requires enhancing genetic, temporal, and spatial diversity (Ditzler et al., 2021).

The agricultural technique of intercropping, involving the simultaneous cultivation of multiple crops in close proximity, holds potential for augmenting crop diversity. Crop diversification contributes to increased yield through the strategic exploitation of factors such as niche differentiation and optimal resource utilization (Li et al., 2020; Yu et al., 2015).

Recent meta-analyses suggest that intercropping enhances yield by around 30% compared to monocultures (Beillouin et al., 2019; Li et al., 2023).

Intercropping has the potential to alleviate pest outbreaks by disrupting pest host-searching behavior (Finch and Collier, 2000; Mansion-Vaquié et al., 2020), diminishing host plant concentration (resource concentration hypothesis) (Root, 1973), or augmenting the abundance, diversity, and control efficiency of natural enemies (natural enemies hypothesis) (Khan et al., 1997; Nilsson et al., 2016; Tajmiri et al., 2017; Trenbath, 1993).

Comprehensive studies have elucidated the impact of genetic and abiotic factors on cabbage yield and crucial traits. Parameters such as genotype, planting density, season, and planting date exert influence on total and marketable yield, head weight, shape, firmness, as well as core dimensions (de Moel and Everaarts, 1990; Fornaris-Rullan et al., 1989; Howe and Waters, 1994; Strandberg and White, 1979).

Specifically, de Moel and Everaarts (1990) observed smaller, lighter heads and reduced marketable yield in crops planted in June and July compared to those planted in May, with an associated increase in core length in the Netherlands.

Fornaris-Rullan et al. (1989) documented head weight variations from 0.63 to 1.73 kg, diameters ranging from 12.4 to 18.6 cm, and lengths spanning 13.8 to 16.3 cm among 10 cabbage cultivars cultivated in Puerto Rico.

Howe and Waters (1994) highlighted substantial year × cultivar interactions affecting marketable yield, head weight, size, and other characteristics across 16 cabbage cultivars planted in two seasons in Florida. Similar variations among cultivars in significant traits were observed in Louisiana, North Dakota, and Pennsylvania (Greenland et al., 2000; Orzolek et al., 2000; Sundstrom and Story, 1984).

Sowing and planting dates vary by geographical regions. In the high hills of India, seeds are sown in May - June for summer/ autumn harvests. In hilly areas with abundant rainfall, planting is limited, occurring only in autumn. In the northern regions, sowing is recommended between August and November for late varieties. In the eastern regions, sowing typically begins in mid to late September (Tnau, 2017; Gupta et al., 2020).

Recommended row spacing for white cabbage in USA ranges from 60 cm to 90 cm, with plant spacing of 22 cm to 38 cm for early plantings and 22 cm to 45 cm for late plantings, based on variety traits, soil fertility, and intended cultivation purpose (Kemble et al., 2018).

In Romania, autumn cabbage crops are established both through seedlings and by direct sowing. Seedlings are transplanted between June 15 and July 15. The planting is done in two rows, with a spacing of 70 cm to 80 cm between rows and 25 cm of 30 cm between plants within a row, resulting in an approximate density of 45.000 to 50.000 plants per ha^{-1} (Munteanu, 2019).

Hence, further research on cabbage growth is imperative, particularly in the face of contrasting and constantly changing climatic conditions. By analysing the morphological characteristics of cabbage plants during the vegetative phase and exploring relationships among key head traits.

Anticipated outcomes include an enhanced comprehension of the effects of planting date and densities on indicators of cabbage quality in the northeastern part of Romania, leading to more efficient cultivar development, evaluation, and selection.

This research aims to contribute to the scientific insights of the white cabbage cultivation and to provide practical implications for refining planting strategies.

This is achieved through the determination of the optimal sowing period for acquiring high-quality seedlings by evaluating their morphological and physiological traits.

Furthermore, the study seeks to identify the optimal combinations of experimental factors, including planting time and density, to attain superior cabbage mother-plants. This experiment is a component of a more comprehensive study dedicated to improving cabbage seed production technology.

MATERIALS AND METHODS

Plant material

The study is centered on the assessment of the vegetative phase of the late-season white cabbage cultivar, ‘Silviana’ patented at the Vegetable Research and Development Station Bacau in 2014. ‘Silviana’ cultivar is characterized as a sturdy and crack-resistant cabbage variety with a good yield potential. The cabbage head displays uniformity and exhibits a range from round to elliptical in longitudinal section, featuring a height between 19-22 cm and a diameter of 22-25 cm. The calculated shape index falls within the range of 0.8-0.95, and the average weight varies from 1.7-3.3 kg. The leaves possess a delicate texture, while the head form in longitudinal section ranges from round to elliptical, with leaves displaying hues from raw green to medium intensity green. The cultivar ‘Silviana’ exhibits the potential to achieve elevated yields ranging from 100 to 120 tons per hectare.

Site description

The studies were conducted in the VRDS experimental field, located at coordinates 46.585205 N, 26.950087 E. The field is located in the north-eastern region of Bacau, situated on a river terrace with an elevation of 5-7 meters and a height of 165 meters above sea level. Positioned in the Bistrița - Siret interfluve, approximately 4 kilometres north of their confluence, the trial took place on a well-developed, loamy-sandy textured polished cambic chernozem.

Experimental design

The experiment involved a randomized complete block design with three replications. For the study, a methodology encompassing morphological observations, biometric determinations, and physiological studies were employed. The cabbage seeds were sown in three distinct dates and maintained in a greenhouse while receiving regular watering, typically once per day, through overhead misters until field planting according to the following periods. The initial planting took place on July 10th (E_1), followed by the second on August 10th (E_2), and the third on September 10th (E_3). Each planting period involved the establishment of three distinct planting densities.

The highest density was achieved with a spacing of 25×140 cm between plants and rows, resulting in an average of approximately 28.500 plants ha^{-1} (D_1). The medium density was attained with a spacing of 40×140 cm, yielding an average of approximately 17.850 plants ha^{-1} (D_2). The lowest density was achieved with a spacing of 55×140 cm, resulting in an approximate of 12.900 plants ha^{-1} (D_3).

After reaching 40 days and achieving planting maturity, the cabbage seedlings that had not been planted were extracted from the greenhouse and transported to the laboratory for analysis. Plant samples were randomly hand-picked for the assessment of various morphological traits.

Determination of assimilatory pigments

The seedling quality was assessed through the determination of assimilatory pigments. Physiological studies were conducted for chlorophyll A, chlorophyll B, chlorophyll A+B, chlorophyll A/B ratio, carotenes, xanthophylls, and the ratio of total chlorophyll/carotenes and xanthophylls. The spectrophotometric method was employed to evaluate these pigments, utilizing the BOECO S-20 Spectrophotometer. For pigment extraction, a one-gram leaf sample (fresh plant material) was extracted with 80% acetone, and the absorption of the extracts was measured at wavelengths 663, 646, and 470 nm compared to the control sample – acetone 80%. Pigment content was calculated following the formulas developed by Mackinney (1941) and expressed in mg 100 g^{-1}.

$$Chlorophyll\ A = [(12.21 \times DO_{663}) - (2.81 \times DO_{646})] \times 5$$
$$Chlorophyll\ B = [(20.13 \times DO_{646}) - (5.03 \times DO_{663})] \times 5$$
$$Carotenes\ and\ xanthophylls = [(1000 \times DO_{470}) - (3.27 \times Chl.A) - (1.04 \times Chl.B)] \div 229$$

Data collection

The evaluation of the seedlings characteristics included the thickness of the seedling at the stem base (mm), the height of the seedling at planting maturity (cm), number of leaves, total seedling weight (g), weight of aerial part of the seedling (g) and roots weight (g). Regarding the study of the morphological features of cabbage plants during the vegetative phase, assessments were conducted on parameters such as head diameter (cm), head height (cm), leaf count and plant height (cm).

Data analysis

The acquired morphological data resulted from the observation of the dynamics in plant growth and developmental processes. Statistical-mathematical methods, including the ANOVA method and Tukey's post-hoc test, were applied to process the obtained data, with reported results presented as means ± standard errors. Software tools such as Microsoft Excel and IBM SPSS Statistics version 26.0 were utilized for data analysis. A significance threshold of $P \leq 0.05$ was employed to discern significant differences between data groups.

RESULTS AND DISCUSSIONS

Table 1 provides data on variations recorded from pigment analyses of cabbage leaves, conducted on three seedling sets, with each set representing a period of establishing the mother plant crop.

Table 1. Results regarding the analysis of pigments in the seedling leaves (mg 100 g^{-1} fresh plant material)

V	Chl. A	Chl. B	Chl. A+B
E_1	14.58±1.12 b	14.48±1.58 c	29.06±2.66 c
E_2	16.27±0.82 b	23.58±0.97 b	39.86±1.51 b
E_3	23.32±0.42 a	28.48±0.97 a	51.80±1.38 a
V	**CX**	**A/B**	**AB/CX**
E_1	7.08±0.41 b	1.05±0.06 a	4.03±0.17 c
E_2	6.90±0.38 b	0.69±0.03 b	5.87±0.27 a
E_3	10.26±0.22 a	0.82±0.01 b	5.04±0.05 b

E_1 - 10.07; E_2 - 10.08; E_3 - 10.09; V - Variant; Chl. A - Chlorophyll A; Chl. B - Chlorophyll B; Chl. A+B - Total Chlorophyll; CX - Carotenes and xanthophylls; A/B - Chlorophyll ratio; AB/CX - Total Chlorophyll/ Carotenes and Xanthophylls ratio; a - the highest value. The values represent the mean ± standard error, and letters indicate significant differences between variants according to the Tukey test for $p \leq 0.05$.

The analysis of experimental data regarding chlorophyll pigments (A) content indicates that the mean varied between 14.58 mg 100 g^{-1} and 23.32 mg g^{-1}. The maximum value was recorded for seedlings from the third planting period, while the minimum was observed for the first period.

In the case of chlorophyll (B), the mean ranged from 14.48 mg 100 g^{-1} fresh plant material to 28.48 mg 100 g^{-1}. The maximum value was obtained in the third period, and the minimum was recorded for the second period.

Concerning total chlorophyll pigments (A+B), the maximum value of 51.58 mg 100 g^{-1} was recorded for the planting date of 10.09, corresponding to the third period. Therefore, the average content of carotenes and xanthophylls (CX) reached a maximum of 10.26 mg 100 g^{-1} fresh plant material for seedlings from the third period.

Regarding the chlorophyll ratio (A/B), the highest mean was recorded during the first period, reaching 1.05 mg 100 g^{-1}. As for the total chlorophyll/carotenes and xanthophylls ratio (AB/CX), the average ranged from 4.03 mg 100 g^{-1} to 5.87 mg 100 g^{-1}, with the highest value recorded during E_2 (10.08).

Results from cabbage seedling analysis showed significant variation between periods. Overall, the values indicate the health, photosynthetic capacity, and adaptability of cabbage seedlings to the sowing date, offering essential insights into physiological processes during the initial plant life cycle stage.

Table 2 summarizes variations in morphological characteristics among the three sets of 40-day-old seedlings, each corresponding to the specific planting period for mother-plant crop. The main objective was to identify the optimal sowing time for quality seedling production.

Table 2. Results regarding the analysis of morphological characteristics of the seedlings

V	TSB (mm)	HS (cm)	NL
E_1	2.05±0.06 b	11.66±0.67 c	5.10±0.23 ns
E_2	2.44±0.10 a	14.66±0.35 b	4.63±0.15 ns
E_3	2.37±0.12 ab	20.47±0.55 a	5.09±0.21 ns
V	**SM (g)**	**MAS (g)**	**RM (g)**
E_1	2.85±0.18 b	2.75±0.19 b	0.107±0.004 b
E_2	2.98±0.09 b	2.86±0.10 b	0.120±0.005 b
E_3	5.09±0.21 a	4.91±0.21 a	0.176±0.008 a

E_1 - 10.07; E_2 - 10.08; E_3 - 10.09; V - Variant; TSB - Thickness of the seedling at the stem base; HS - Height of the seedling at planting maturity; NL - Number of leaves; SW - Total seedling mass; WAS - Mass of aerial part of the seedling; RW - Roots mass; a - the highest value; ns - not significant. The values represent the mean ± standard error, and letters indicate significant differences between variants according to the Tukey test for $p \leq 0.05$.

The analysis of seedling thickness at the stem base revealed that the maximum mean was recorded in E_2 (10.08) with a diameter of 2.44 mm, while the E_1 (10.07) showed an average of 2.05 mm. Regarding the comparison of seedling planting height across all periods, the highest average height was recorded in E_3 with a value of 20.47 cm, and the lowest was recorded E_1 with a value of 11.66 cm.

Leaf counts showed minimal, insignificant variations, ranging from 4.63 in E_2 to an average of 5.10 leaves per plant, with the highest mean recorded by E_1. The total mass of seedlings exhibited significant variations across periods, ranging from a minimum of 2.85 g in E_1 to a maximum of 5.09 g in E_3. Similarly, the mass of aerial part showed a range from 2.75 g to 4.91 g, with E_3 recording the highest value and E_1 the lowest.

Root mass averages showed significant variation, ranging from 0.107 g to 0.176 g, with E_3 recording the highest and E_1 the lowest means.

Morphological analysis revealed significant differences between sowing periods, suggesting the E_3 is potentially the most suitable for high-quality seedling development. However, these findings are limited and warrant further study for a comprehensive understanding of the sowing period's impact on cabbage seedling development.

Table 3 presents observed differences in morphological characteristics, including plant height, leaf count, head diameter, and head height, concerning the effect of the establishment period on mother-plants crop quality.

Table 3. Results regarding the analysis of the influence of the planting dates on mother-plants

V	NL	PH (cm)
E_1	24.68±0.41 a	56.70±0.87 a
E_2	22.96±0.30 b	49.05±0.88 b
E_3	19.90±0.50 c	37.51±0.90 c
V	**HD (cm)**	**HH (cm)**
E_1	23.30±0.38 a	19.14±0.35 a
E_2	18.53±0.43 b	15.49±0.34 b
E_3	-	-

E_1 - 10.07; E_2 - 10.08; E_3 - 10.09; V - Variant; NL - Number of leaves; PH - Plant height; HD - Head diameter; HH - Head height; a - the highest value. The values represent the mean ± standard error, and letters indicate significant differences between variants according to the Tukey test for $p \leq 0.05$.

Plants established in E_1 had a higher leaf count compared to those in E_2 and E_3, with the maximum mean of 24.68 cm and the minimum mean of 19.90 cm recorded by E_3 (10.09). The first period (10.07) proves to be the most conducive for leaf system development.

Regarding plant height, there is a notable variation among those plants that have been established in E_1, with an average of 56.70 cm, compared to those in E_3 which recorded an average of 37.51 cm.

The head diameter and head height could only be analysed for plants established in the first and second periods because the plants from the third period did not reach the head-forming stage until they were covered with soil for winter protection in preparation for the upcoming seed crop. Consequently, both head diameter and height showed significantly higher averages for plants analysed in the first period compared to the second period.

It has been highlighted that the planting date influences head characteristics. In their study, Kleinhenz and Wszelaki (2003) reported no differences in cabbage head length and width for crops planted in May compared to those planted in June-July in the state of Ohio. De Moel and Everaarts (1990) emphasized that plants cultivated in June and July had smaller and lighter heads compared to those cultivated in May, observing an increase in head length with later planting in the Netherlands.

The results of the analysis on the influence of planting densities on morphological characteristics in the vegetative phase are presented in Table 4.

Table 4. Results regarding the analysis of the influence of densities on mother-plants

V	NL	PH (cm)
D_1	21.68±0.50 ns	49.47±1.58 ns
D_2	22.98±0.53 ns	47.53±1.28 ns
D_3	22.88±0.42 ns	46.25±1.28 ns
V	HD (cm)	HH (cm)
D_1	20.11±0.61 ns	16.87±0.54 ns
D_2	21.57±0.52 ns	17.60±0.46 ns
D_3	21.07±0.75 ns	17.47±0.56 ns

D_1 - 28.500 plants ha^{-1}; D_2 - 17.850 plants ha^{-1}; D_3 - 12.900 plants ha^{-1}; V - Variant; NL - Number of leaves; PH - Plant height; HD - Head diameter; HH - Head height; ns–not significant. The values represent the mean ± standard error, and letters indicate significant differences between variants according to the Tukey test for $p \leq 0.05$.

From the analysis of the obtained data, it can be observed that the number of leaves were not significantly influenced by the planting densities. The means ranged between 21.68 and 22.98 leaves per plant, with values very close to each other. The maximum mean was recorded for the density of 17.800 plants ha^{-1}, while the minimum mean was recorded by the density of 25 × 140, equivalent to 28.500 plants ha^{-1}.

Regarding plant height, the obtained values varied insignificantly. As expected, the D_1 with 28.500 plants ha^{-1} recorded the highest average height, reaching 49.47 cm. With increasing plant density, the plants tend to elongate in search of the necessary light for physiological processes.

Regarding head diameter, the averages varied insignificantly. The maximum average of 21.57 cm was achieved with by the density of 17.850 plants ha^{-1}, while the minimum average of 20.11 cm was recorded with a density of 28.500 plants ha^{-1}. Semuli (2005) noted that reducing the distance between plants increases competition for nutrients, light, air, and humidity, potentially leading to a decrease in head diameter and weight. Stoffella and Fleming (1990) reported increases in head height and width with greater spacing between plants in rows, ranging from 8 cm to 38 cm. The smallest distance between plants resulted in a significantly larger equatorial diameter than the polar diameter.

The planting density did not significantly impact head height. Heights ranged from 16.87 cm to 17.60 cm, with the maximum mean recorded at a density of 17.850 plants ha^{-1} and the minimum at 28.500 plants ha^{-1}.

Overall, these findings suggest that the planting density used does not significantly influence the morphological characteristics of white cabbage parent plants. However, other important variables that may affect plant development should also be considered, which could vary depending on the planting density.

The outcomes of the analysis regarding the influence of planting time and density on morphological traits are displayed in Table 5.

The assessment of head diameter and height was limited to plants cultivated in the first and second periods, as those from the third period had not reached the requisite developmental stage for head formation at the time of soil covering. Following the analysis for the experimental data for leaf number per plant, a notable mean variation is found, ranging from 17.94 to 25.38. The lowest value, 17.94, occurred for the $E_3 \times D_1$ variant (10.09 × 28.500 plants ha^{-1}), and the highest value, 25.38, was observed for the E_1 (10.07) with D_2 (17.850 plants ha^{-1}) combination.

Table 5. Results regarding the analysis of the combined influence of planting dates and densities on mother-plants

V	NL	PH (cm)
$E_1 \times D_1$	24.33±0.67 ab	59.05±1.39 a
$E_1 \times D_2$	25.38±0.71 a	55.43±1.51 ab
$E_1 \times D_3$	24.33±0.77 ab	55.62±1.58 ab
$E_2 \times D_1$	22.77±0.51 abc	51.82±1.71 bc
$E_2 \times D_2$	23.38±0.62 ab	49.86±1.12 bc
$E_2 \times D_3$	22.72±0.46 abc	45.48±1.36 c
$E_3 \times D_1$	17.94±0.59 d	37.56±2.10 d
$E_3 \times D_2$	20.16±0.99 cd	37.31±1.35 d
V	HD (cm)	HH (cm)
$E_1 \times D_1$	22.47±0.60 ab	18.77±0.61 ab
$E_1 \times D_2$	22.77±0.76 ab	18.61±0.64 ab
$E_1 \times D_3$	24.66±0.55 a	20.03±0.54 a
$E_2 \times D_1$	17.75±0.75 cd	14.98±0.65 c
$E_2 \times D_2$	20.37±0.62 bc	16.58±0.58 bc
$E_2 \times D_3$	17.48±0.71 d	14.90±0.50 c
$E_3 \times D_1$	-	-
$E_3 \times D_2$	-	-
$E_3 \times D_3$	-	-

$E_1 \times D_1$ - 10.07×28.500 plants ha^{-1}; $E_1 \times D_2$ - 10.07×17.850 plants ha^{-1}; $E_1 \times D_3$ - 10.07×12.900 plants ha^{-1}; $E_2 \times D_1$ - 10.08×28.500 plants ha^{-1}; $E_2 \times D_2$ - 10.08×17.850 plants ha^{-1}; $E_2 \times D_3$ - 10.08×12.900 plants ha^{-1}; $E_3 \times D_1$ - 10.09×28.500 plants ha^{-1}; $E_3 \times D_2$ - 10.09×17.850 plants ha^{-1}; $E_3 \times D_3$ - 10.09×12.900 plants ha^{-1}; V - Variant; NL - Number of leaves; PH - Plant height; HD - Head diameter; HH - Head height; a - the highest value. The values represent the mean ± standard error, and letters indicate significant differences between variants according to the Tukey test for $p \leq 0.05$.

Regarding plant height, a notable mean variation was detected, ranging from a minimum average of 37.31 cm for E_3 (10.09) with D_2 (17.850 plants ha^{-1}) to a maximum average of 59.05 cm for E_1 (10.07) with D_1 (28.500 plants ha^{-1}).

Regarding head diameter, significant differences are noticeable among the mean values of the studied variants. The $E_1 \times D_3$ experimental variant consistently achieved the maximum values, at 24.66 cm, while the $E_2 \times D_3$ variant recorded the minimum average at 17.48 cm. Head height displayed significant variation of mean values, with the $E_1 \times D_3$ variant (10.07×12.900 plants ha^{-1}) reaching the maximum mean of 20.03 cm and the $E_2 \times D_3$ variant (10.08×12.900 plants ha^{-1}) registering the minimum mean at 14.90 cm.

Abed et al. (2015) reported that the interaction between planting date and plant density had no significant impact on cabbage head characteristics. Variations in head width, height, volume, and firmness were ascribed to the independent effects of planting date or plant density.

Results indicate that both planting date and density exerted significant influence on head morphology, particularly in terms of length and width. The earliest planting period, coupled with the lowest density, returned the greatest means for head length and width. Consequently, plants sown during D_1 (10.07) exhibited distinctly larger heads compared to those planted later, such as during D_3 (10.09), which did not reach the stage of head formation.

CONCLUSIONS

In the pursuit of determining the optimal sowing period for producing superior seedlings, it was noted that the third period returned the most favourable outcomes, ensuring the attainment of seedlings of superior quality.

In terms of assimilatory pigment content in seedlings, E_3 (10.09) revealed the highest chlorophyll a and b content, while E_2 (10.08) exhibited the highest mean ratio of total chlorophyll to carotenes and xanthophylls.

The study on the influence of the planting period of cabbage plants morphology indicates that the first period (E_1) positively impacted the leaf system, averaging 24.68 leaves per plant. Plant height averaged 56.70 cm, with the highest head diameter and height values also linked to the first period, highlighting its significant positive effect on the evaluated morphological traits.

Regarding density influence, it manifested non-significant variability in the main morphological characteristics of the mother plants, with closely aligned means. Specifically, density D_1 (28.500 plants ha^{-1}) was linked to the maximum height at 49.47 cm, while D_2 (17.850 plants ha^{-1}) demonstrated the highest head diameter values.

In examining the effects of planting period and mother-plant crop density on morphological traits, noteworthy variations emerged across all analysed characteristics. Prominently, combinations such as $E_1 \times D_2$ and $E_1 \times D_3$ demonstrated superior outcomes, exerting a significant positive impact on the development of cabbage morphological traits.

These findings suggest that experimental factors greatly influence plant development, and selecting the optimal factors can notably enhance the morphological traits of white cabbage, fostering the growth of viable mother-plants used for future seed production.

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DETERMINATION OF THE NUTRITIONAL COMPOSITION OF CARROT POMACE

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Abstract

Carrot (Daucus carota L.) is one of the most important root vegetables cultivated worldwide, due to its nutritional valueand the phytochemicals content which promotes health. Carrot root is a rich source of carotene, carbohydrates, fiber, minerals, vitamins and other biologically active compounds. The processing of carrot root involves the production of waste such as carrot pomace, which can represent a good source of important nutritional and bioactive compounds. The objective of this work was to evaluate the nutritional content of carrot pomace resulting as a by-product of obtaining carrot juice from carrots sold in local agro-food markets. The results obtained showed that the analyzed dried pomace carrot contains important amounts of nutritional compounds that vary depending on the origin of the carrot: 49.02-53.16% carbohydrates, 25.61-31.88% fiber, 5.48-6.95% minerals (ash), 6.32-7.34% protein, 0.88-1.36% fat. The highest fiber potential was recorded in the case of samples from the batch cultivated in Dudestii Noi, while carrot samples grown in Timisoara recorded the highest intake in proteins, lipids, minerals and carbohydrates. The values of the nutritional parameters suggest the use of dried pomace carrot to obtain products with added nutritional value. The superior use of carrot pomace, as a secondary product, can be an ecological way of limiting the waste resulting from the processing of carrot roots.

Key words:*carrot pomace, nutritional parameters, carrot roots, by-product valorization, waste.*

INTRODUCTION

Carrot (*Daucus carota* L.) among the most important vegetables root cultivated worldwide have attained popularity due to their nutritional value, being rich in nutrients and health-promoting compounds, including minerals, sugars, carotenoids and phenols (Nazar et al., 2023; Šeregelj et al., 2020). They are used fresh or made into juices, beverages, candies, preserves and dehydrated products. Even if carrot roots come in many varieties that vary with size, shape, and color (such as orange, purple, red, white and yellow), orange carrot is the most popular due to its high α- and β-carotene content that contributes to the provitamin A activity (Kamiloglu et al., 2015). Carrot is a significant source of natural bioactive compounds, including phenolics, and carotenoids, as well as ascorbic acid and tocopherols, being classified as a vitaminized food (Surbhi et al., 2018). Due to the presence of an appreciable level of different compounds, the carrot is considered a functional food with significant health-promoting properties: the prevention and therapy of cardiovascular diseases, cancer, diabetes, gastrointestinal diseases, ocular diseases, etc. (Nazar et al., 2023; Šeregelj et al., 2020). Important quantities of these nutritional and bioactive compounds are found in carrot pomace obtained as waste from the processing of carrot roots. On dry weight basis, carrot pomace contains: 4-5% protein, 8-9% reducing sugar, 5-6% minerals, 37-48% dietary fiber, 3.2 mg/g Na, 18.6 mg/g K, 1.8 mg/g P, 3.0 mg/g Ca, 1.1 mg/g Mg, 4.0 mg/g Cu, 10.8 mg/g Mn, 30.5 mg/g Fe, 29.4 mg/g Zn and important contents of vitamin B-complex, vitamin A, tocopherols, and ascorbic acid (Šeregelj et al., 2020). The recovery of these nutritional and bioactive compounds can generate functional ingredients and provide utilization of carrot waste in different food products that confer beneficial effects on human health (Ikram et al., 2024; Nazar et al., 2023; Šeregelj et al., 2020). Previous studies have shown that the composition of carrot pomace, has been studied by many researchers. The results obtained by different researchers who analyzed the nutritional composition of carrot pomace resulting from the processing of carrot root, will be presented below. Important data regarding the composition of carrot pomace were presented by determining the nutritional profile of carrot pomace powder on fresh/dry weight basis: 83.40 ± 0.23% moisture, 5.83 ± 0.25% protein, 6.17 ± 0.08% ash, 52.73 ± 0.01% total carbohydrates, 42.59 ± 0.01% total dietary fiber. Carrot pomace powder is a promising source of polyphenols and prebiotics for improving good health (Mall and Patel, 2024). Carrot pomace comprises about 4-5% protein, 8-9% sugar, 5-6% minerals and 37-48% total dietary fiber, so the carrot product is a rich source of dietary fiber. In the same study, the dried carrot pomace contains 2.5% moisture, 5.5% ash, 1.3% fat, 0.7% protein, 20.9% crude fiber, 55.8% dietary fiber, 71.6% total carbohydrate (Ikram et al., 2024). Proximate composition of carrot pomace was also determined by Begum et al. (2023) who reported that carrot pomace powder used to obtain the assortment of bread with added nutritional value, contains 9.06% moisture, 6.71% ash, 1.19% raw fat, 13.09% raw fiber , 63.70% raw carbohydrate, 6.46% raw protein, 11.83 mg/100 g β-carotene, 1.53 mg/100 g vitamin C. In a study that propose to characterize the carrot pomace powders from four varieties (Baltimore, Niagara, Belgrado and Sirkana) it was found that carrot pomace powders had high contents of fiber, carbohydrates, ash, and proteins: 20.09-33.34%, 46.55-58.95%, 5.29-5.89%, respectively 6.87-9.14% (Luca et al., 2022). The analysis of carrot pomaces resulting from carrot processing into juice found that the powder of this pomace contains 7.12-7.55% moisture, 6.84-7.28% ash, 6.55-6.89% protein, 1.46-1.78% fat, 47.80-51.67% total fibers and 13.65-16.85% total sugar. The nutritional composition of carrot pomace allowed its use to obtain biscuits with added value. Considering the carrot and pepper waste (damaged, rotten, non-edible parts) the following nutritional parameters was found: <0.01% free fat, 1.52% crude protein, 6.34% total dietary fiber, 8.8% digestible carbohydrate, 7.22% total sugars, 5.24% reducing sugars, 18.8 dry matter and 2.11% total ash (Zhivkova, 2020). During an investigation on the potential of carrot pomace utilization, Sahni and Shere (2017) discover that powder of carrot pomace has the following proximate composition: 6.82% moisture, 1.44% fat, 11.12% raw fiber, 9.85% ash, 15.85% protein, 54.91% carbohydrates. Nutritional profile of carrot pomace reveals the fact that it is a cheap source of dietary fiber, thus justifying its use for fiber enrichment in food products. A high fiber bread was prepared by replacing maximum 4% Maida (super-refined wheat flour used in Indian cuisine to make pastries and other bakery) with carrot peel powder has the following nutritional composition: 8.10% moisture, 5.51% protein, 2.12% fat, 24.06% carbohydrate, 65.16% fiber, 3.15% ash. Study on the nutritional composition, and shelf stability of carrot pomace-incorporated cookies, noted that the incorporation of carrot pomace powder containing 6.54% moisture, 6.50% protein, 14.75% soluble fiber, 30% insoluble fiber, 5.12% ash, 5.456 μg/100 g total carotenoid, 607 μg/100 g β-carotene, contributes with a significant amount of micronutrients and fiber making the product very nutritious (Bellur & Prakash, 2015). The nutritional composition of carrot peels was determined being investigated the effect of adding carrot peel fibers and vitamin A on iron bioavailability in biscuits: 12.47% moisture, 6.09% protein, 2.05% fat, 9.87% fiber, 10.48% ash, 71.5% carbohydrates, 7136.06 μg retinol equivalent (RE)/100 g vitamine A (Khalil et al., 2011). Important values of the nutritional content of carrot pomace attest that these fluor is a good source of dietary fiber: 6.80% moisture, 5.26% ash, 4.93% total protein, 73.00% total dietary fiber, 15.38% soluble dietary fiber and 57.62% insoluble dietary fiber having a great potential to be used as functional ingredients in cookie formulations and to improve nutraceutical properties of cookies (Turksoy & Özkaya, 2011). From those presented above it can be observed that carrot pomace, resulting from the processing of the carrot root, still contains important amounts of nutritional compounds, which it varies within fairly wide limits. This can be explained by the different maturity and varieties, as well as by the different ratios of the anatomical parts in the studied products

(Iegorov et al., 2019). It can also be seen that the residual material left after carrot processing, usually resulting from carrot juice extraction or other processing methods, is a nutritionally rich by-product. The objective of this work was to evaluate the nutritional content of carrot pomace resulting as by-product for obtaining carrot juice from carrots sold in local agricultural markets.

MATERIALS AND METHODS

The analyzed material was composed from the residue (peel + pulp) resulting as waste from obtaining three batches of carrot juice using a fruit juicer. The notion of carrot pomace (identified as CP) designates the material consisting from the carrot peel resulting from peeling and the carrot pulp left after the preparation of carrot juice. The carrot roots were taken from three different agro-food markets located in Timisoara (Romania) and come from local producers in the area bordering Timisoara. Three groups of carrot samples were made up, marked in the text with L1 (cultivated in Timisoara), L2 (cultivated in Varias), L3 (cultivated in Dudestii Noi). For each group were chosen, random between 600-700 g carrot root. Before use, the carrot roots were cleaned by washing with tap water, rinsed with distilled water and dried by blotting with filter paper. The solid pulp left after squeezing the juice together with the peel resulting from peeling the carrot root (Figure 1) were dried in an oven at 60°C for 24 hours.

Figure 1. Fresh carrot pomace: peel and pulp, remaining after squeezing the carrot juice

The dried pulp and peel (Figure 2) were homogenized and ground using a kitchen grinder.

Figure 2. The dried carrot pomace: the peel and pulp

The homogeneous dry powder obtained in this way (Figure 3), corresponding to the carrot lots: L1, L2 and L3, was used to determine the concentrations of the nutritional parameters.

Figure 3. Dried carrot pomace powder

Determination of nutritional parameters of carrot pomace: moisture, fat, protein, ash, were performed in accordance with the AOAC (2006) method guidelines. AOAC method no. 925.05 was used to determine fat and ash, while methods no. 925.10 and no. 925.36 were used to determine moisture and protein content respectively. Briefly, moisture contents were determined by drying in the drying chamber (BINDER GmbH, Tuttlingen, Germany) at 105 °C up to a constant mass. Ash content of dried carrot pomace was determined, through the calcination method, at 550°C usingthe calcination furnace (Nabertherm Lilienthal, Germany). Protein content was determined by the Kjeldahl method using a KjeltecTM 8400/8420/8460 FOSS equipment. Digestion of the sample was carried out with sulfuric acid and potassium sulfate. After digestion and dilution with water, the sample was distilled in alkaline medium (NaOH), thus releasing ammonia which was quantified by titration with a standardized acid solution in the presence of colorimetric indicators methyl red and bromocresol green. The crude fat was determined using the Soxhlet method using hexane as extraction solvent and Soxtest Raypa SX-6 MP equipment at 75°C and an extraction time of 50 minutes. Crude fibers were determined by using the Foss method (Fibertec™ 2010 Automated Crude & Detergent Fibre Solution) according AOAC 978.10 procedure. The method involves crude fibre determination by boiling the sample in acid hydrolysis with 1.25% H_2SO_4 for the extraction of sugars and starch, followed by alkaline hydrolysis with 1.25% NaOH, which removes proteins. and some hemi-cellulose and lignin. The organic substance remaining after this treatment is the crude fiber. The carbohydrate content was calculated by difference, applying the equation: Carbohydrates (%) = 100 - (protein + fat + ash + fiber + moisture) (Luca et al., 2022). The determinations were made in triplicate.

RESULTS AND DISCUSSIONS

The results obtained for the determination of moisture, ash, protein, fat, fiber and carbohydrate concentrations from the analyzed carrot pomace samples are presented in Table 1. The data presented in this table show that the analyzed dried pomace carrot contains important amounts of nutritional compounds which varies depending on the provenience of the analysed carrot lots and the nature of the analyzed parameter. The moisture content of carrot pomace provides data on the water content of the analyzed product,respectively the organic and inorganic dry substance content. A higher moisture content contributes to decreasing of the storage period, respectively to the loss of pomace powders quality attributes. The moisture content of carrot pomace, experimentally determined have low contents, between 6.28-7.12%. These values allow preservation, under normal weather conditions, for an acceptable period of time. The highest content was determined in L2 group, and the lowest in L1. Comparing the carrot pomace moisture values obtained experimentally with those reported in previous studies, it can be seen that they are comparable to those reported: 7.6% - in pomace powder, in the range 7.12-7.65% - in powders from carrot wastes, 6.80% - in carrot peel powder, 6.64% - in carrot pomace (Sahni and Shere, 2017; Luca et al., 2019; Turksoy and Özkaya, 2011; Bellur and Prakash, 2015). Lower humidity values were obtained: 3.78-5.91% - in carrot pomace powders, respectively 2.5% - in carrot pomace (Luca et al., 2022; Nazar et al., 2023).

The composition of carrot root,, reported by other authors, highlighted the following values: 9.58 g/100 g carbohydrates, 0.93 g/ 100 g protein, 0.24 g/100 g fat, 2.80 g/100 g fiber, 4.74 g/100 g total sugars (of which 3.59 g/100 g sucrose, 0.59 g/100g glucose and 0.55 g/100 g fructose (Ikram et al., 2024).

The ash content, respectively the inorganic matter content is a measure of the mineral amount contained in the analyzed product (Velciov et al., 2022). A high ash content shows increased concentrations of essential mineral elements that have numerous health benefits including tissue maintenance, bone and teeth formation and health, serving as cofactors and coenzymes to enhancing various enzyme systems, adding the regulation and coordination of most body functions, and other biochemical and physiological functions (Godswill et al., 2020). The carrot pomace samples taken in the experiment show important mineral contents, presenting values between 5.48-6.95%. It can be observed that the highest ash concentrations were determinated in the L1 group (6.95 ± 0.60%) and the small ones in the L2 group (5.48 ± 0.45%). These values confirm that carrot pomace is a valuable source of macro and microelements (Ikram et al., 2024; Surbhi et al., 2018). The analyzed carrot pomace could be used as an addition in foods that it improves their nutritional quality and completes the content of mineral elements such as manganese, iron, potassium and zinc. Comparing the obtained values with those reported by other authors it can be seen that

Table 1. The concentrations of nutritional parameters of carrot pomace powder (mean values in triplates)

Specification	Parameter values (%)					
	Moisture	Ash	Protein	Fats	Fibers	Carbohydrate
Group 1 (L1)	6.28±0.40	6.95±0.60	7.34±0.80	1.36±0.16	25.61±1.521	53.16±1.87
Group 2 (L2)	7.12±0.53	5.48±0.45	6.32±0.49	0.88±0.12	31.88±1.52	49.02±1.31
Group 3 (L3)	6.52±0.42	6.34±0.49	6.45±0.51	1.23±0.19	29.76±1.33	52.11±1.85

they are comparable to those reported: 5-6% - in carrot pomace (Ikram et al., 2024) ; 6.17% - in carrot pomace powder (Mall and Patel, 2024); 6.71% - in carrot pomace powder (Begum et al., 2023) ; between 5.29-5.89% - in carrot pomace powders (Luca et al., 2022); 6.84-7.78% - in powders from carrot wastes (Catana et al., 2019) and 6.38% – in carrot pomace (Sahni and Shere, 2017). Lower values of the ash content: 3.15% - in carrot pomace powder and 5.12% - in carrot pomace were determined (Kamaliya et al., 2020; Bellur & Prakash, 2015). Higher values 7.29% and much higher 10.48% were reported by other authors (Ikram et al., 2024; Khalil et al., 2011). **Proteins,** the main source of amino acids and nitrogen, are irreplaceable nutrients for human food because they are involved in all physiological functions (Ivanović et al., 2023). Although most plant proteins provide the necessary amounts of essential amino acids for human needs, plant proteins are often recognized as incomplete or nutritionally inferior to animal proteins (Sá et al., 2020). However, the protein contents determined in the analyzed carrot pomace are valuable compounds in human nutrition. The analyzed carrot pomace show values between 6.32-7.34%. Highest protein percentage values were find out in L1 (7.34 ± 0.80%), lower values were determined in groups L3 and L2 (6.45 ± 0.51% and 6.32 ± 0.49%). These values are close to those established: 6.46% - in carrot powder, 6.87-9.14% – in carrot pomace powder, 6.55-6.89% in carrot powder wastes and 6.50% - in carrot pomace powder (Begum et al., 2023; Luca et al., 2022; Catană et al., 2019; Bellur et al., 2015). Lower values were found out: 5.83% - in carrot pomace powder, 0.7% - in carrot pomace, 6.09% and respectively 4.93% - in carrot pomace powder (Mall et al., 2024; Nazar et al., 2023; Kamaliya et al., 2020; Turksoy and Özkayan, 2011). The protein content, as well as other nutritional and biologically active compounds, make it possible to use the analyzed carrot pomace powder to fortify bakery and pastry products. Fat**,** a dietary macronutrient, gives foods their unique flavor and texture. This essential macronutrient, the most energy dense nutrient, helps absorb and transport carotenoids and fat-soluble vitamins being an essential component of cell membranes (Velciov et al., 2022). Even if the analyzed carrot pomace has lower fat contents, they are used as an additive to improve the formulation of cakes (Ikram et al., 2024). The analyzed samples of carrot pomace powder contain low and relatively close amounts of fats, the values being between 0.88-1.36%. The values of the fat content obtained in the analysis of carrot pomace taken in this study are lower than: 2.12% - in carrot pomace powder, 3.48% - in carrot pomace, 2.05% - in carrot peel (Kamaliya et al., 2020; Sahni and Shere, 2017; Khalil et al., 2011). Values close to those obtained in this study were determined: 1.3% - in carrot pomace, 1.19% - carrot pomace, through 0.70-1.13% - in carrot pomace powder and have slightly lower values than those obtained between 1.46-1.78% - in carrot powder wastes (Nazar et al., 2023; Begum et al., 2023; Luca et al., 2022; Catana et al., 2019). Dietary fibers are the edible parts of fruits and vegetables that are resistant to hydrolysis by digestive enzymes (Šeregelj et al., 2020). Dietary fibers are necessary for the body, because they help reduce the risk of many cancers, cardiovascular diseases and other gastrointestinal problems (Nazar et al., 2024). Carrot pomace is an excellent source of dietary fiber, soluble and insoluble fibers, which contributes to improved digestive health and satiety (Ikram et al., 2024). On a dry weight basis, the composition of dietary fiber includes carrot pomace as cellulose (51.6%), lignin (32.1%), hemicellulose (12.3%), and pectin (3.88 %) (Nazar et al., 2024). The results obtained from the analysis of carrot pomace (Table 1) showed increased amounts of dietary fibers, between: 49.02-53.16%. Higher and relatively close values were determined in the L1 and L3 lots (53.16 ± 1.87%, respectively 52.11 ± 1.85%). The values of fiber concentrations in carrot pomace are found between the limits: 28.29-33.34% - in carrot pomace (Luca et al., 2022) but they are smaller than those reported 69.85% - in carrot pomace powder (Ikram et al., 2024), 42.59% - in carrot pomace powder (Mall, et al., 2024), 71.62% - in carrot pomace (Kamaliya et al., 2020) and higher than the value determined: 17.94% in carrot pomace (Sahni and Shere, 2017). These data show that the analyzed carrot pomace powder can be considered as a source of dietary fiber. Carbohydrates are one of the three macronutrients in the human diet, along with protein and fat, playing an important role in energy and plastic metabolism (Barnokhon et al., 2022). They act as an energy source, help control blood glucose and insulin metabolism, participate in cholesterol and triglyceride metabolism, and help with fermentation (Holesh et al., 2023). Previous studies reveal that carrot pomace contains considerable amounts of carbohydrates (Luca et al., 2022). Significant amounts of carbohydrates and relatively close were also identified in the analyzed carrot pomace samples, the concentration limits being between: 25.61-53.16%. It can be observed slightly higher carbohydrate concentrations in pomace carrots group L1 (53.16 ± 1.87%) and L2 (52.11 ± 1.85%) in comparison with the carbohydrate content of L3 group. Comparing the values of the carbohydrate content determined in this study with the values reported by different researchers it can be stated that these are positioned within the range of 46.55-58.95% - in carrot pomace and have lower values than 71.6% (Luca et al., 2022; Nazar et al., 2023). As a conclusion to the experimental results of the nutritional parameters of carrot pomace taken in the experiment, it can be stated that the analyzed carrot pomace powder contains important amounts of fibers, minerals, protein, carbohydrates and low amounts of fat.

CONCLUSIONS

Carrot pomace obtained as a by-product from the industrial processing of carrot roots contains significant amounts of nutritional and biologically active compounds, important for a good health. The experimental results obtained from the analysis of carrot pomace (6.28-7.12% moisture, 5.48-6.95% ash, 6.32-7.3% protein, 0.88-1.36% fat, 25.61-31.88% fiber and 49.02-53.16% carbohydrates) shows that it contains important amounts of nutritional products. The powder of analyzed pomace carrot is characterized by important contents of fibers, minerals, protein, carbohydrates and reduced amounts of fat, depending on the provenience of the analyzed carrot lots and the nature of the analyzed parameter. Group L1, cultivated in Timisoara, was distinguished by the highest content of protein, lipid, carbohydrates and minerals, while group L3, cultivated in Dudestii Noi, is rich in fiber, compared to the other samples analyzed. These nutritional values and the previous scientific research show that carrot pomace obtained from carrot root, after a preliminary processing, can be considered as an additive for improving the quality of some foods, respectively utilized in the development of healthy food. Also, the superior use of carrot pomace, as a secondary product, can be an ecological way of limiting the waste resulting from the processing of carrot roots.

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EFFECT OF APPLICATION OF BIOSTIMULANT PROTIFERT LN 6.5 ON THE EPIPHYTIC AND RHIZOSPHERE BACTERIA OF PEPPER SEEDLINGS

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Abstract

The study estimated the effect of biostimulant Protifert LN 6.5, with either foliar or soil treatment at three different doses - 1%, 2%, and 3% on the epiphytic and rhizosphere microflora of pepper seedlings of two varieties - Kurtovska kapia 1619 (KK) and Bulgarski rotund (BR). Irrespectively of the type of treatment, the higher number of epiphytic and rhizosphere bacteria was observed when the biostimulant was applied at dose of 2% but the effect was dependent on the pepper variety. Analysis showed that the type of treatment significantly affected only the microflora in the area of application. The doses of 1% and 3% did not affect the number of bacteria and in general, the estimated values was lower than those for control plants. The beneficial effect of biostimulants based on amino acids is related to improvement of nutrients absorption and the mineralization of organic matter which can increase soil microorganisms abundance. Further research can focus on mechanism of action of biostimulants and their specific effects on plant growth and productivity and on microflora.

Key words: *biostimulant, epiphytic, pepper, rhizosphere microflora.*

INTRODUCTION

The modern agricultural practices aim not only an increase of plant productivity and yield but also preserving the soil fertility. However, the integral part of most practices is application of chemical fertilizers that according to the aim set by the EU should be reduced with 20 % until 2050. A number of studies have indicated that the soil microorganisms are the most dynamic indicators for agricultural systems and are important indicators of soil fertility (Mamilov & Dilly, 2002; Anderson, 2003). One of the possible alternatives to the widely applied chemical fertilizers are plant biostimulants which are defined as useful natural substances that can improve plant growth and development even at minimal doses (Luta et al., 2024). The category of plant biostimulants is comprised of various bioactive natural substances, such as hydrolysates of animal and plant proteins, which possess the potential to improve crop performance. Chemically, the protein hydrolysates are defined as “mixtures of polypeptides, oligopeptides, and amino acids that are produced from protein sources by partial hydrolysis” (Schaafsma, 2009). According to Calvo et al. (2014) the protein-based plant biostimulants are two types: 1) protein hydrolysates consisting of a mixture of peptides and amino acids of animal or plant origin, and 2) a single amino acids formulation of glutamate, glutamine, proline, etc. The biostimulant Protifert LN 6, which was used in the current study, belongs to the former of the abovementioned groups of protein hydrolysates. Furthermore, the use of protein hydrolysates obtained by animal residues processing can be used as an eco-friendly approach for waste reduction (Colla et al., 2015). The biostimulants have already been applied not only for staple crops aiming to achieve various effects, including stress alleviation, but also for vegetable crops (Salmani and Rezaei, 2023). The pepper is the second most important vegetable crop in Bulgaria, and it is widely grown across the country and is used for different purposes – as fresh, dry, canned food or for spice preparation. A large part of the vegetable crop is grown with pre-produced seedlings which is the main agrotechnological practice in vegetable production. It has been reported that the seedlings germination and plant growth were stimulated by the application of biostimulants and the effects were explained by the action of signaling bioactive molecules that participate in the primary and secondary plant metabolism (Calvo et al., 2014). Positive effects of application of biostimulant Protifert on potatoes have been reported by Ghemam et al. (2016) and on peach by Laita et al. (2022). The studies about the use of biostimulants on vegetable crops and especially about their effects on epiphytic and soil rhizosphere microflora are insufficient. Due to variety of available biostimulant and their versatile effects, there are still many uncertainties about the application times, methods and doses (Shahrajabian et al., 2021). The growing interest about the use of biostimulants demands for more information about their properties and usage.

The objective of the current study was to assess the effect of biostimulant Protifert LN 6.5 on the epiphytic and rhizosphere bacteria during cultivation of pepper seedlings.

MATERIALS AND METHODS

The experiments were conducted in the Experimental Field of the Agricultural University of Plovdiv, Bulgaria and analyses in the laboratories of the Departments of "Horticulture" and "Microbiology and ecological biotechnologies". Seeds from pepper variety Kurtovska Kapia 1619 (KK) and Bulgarian Rotund (BR) were sown in 176-hole styrofoam trays during the first ten days of March at each experimental year. In phase two true leaves, the seedlings were transplanted into pots (number 8) and were grown in an unheated plastic greenhouse. Each of the tested variants has three replications with 15 plants each. The experiments and analysis were done in the period 2021-2023. As soil substrate was used Peat Domoflor Mix 4 UAB Domoflor, Vilnius, Lithuania, with the following characteristics: 100% white peat with particle size 0-10 mm, pH 5.5-6.5, EC 0.6-0.7 mCm/cm, N:P:K - 14:16:18kg.m^3 and perlite in a ratio of 3:1. At the development of four leaves, the foliar fertilizer Protifert LN 6.5 (manufacturer SICIT 2000, Italy) at doses of 1%, 2% and 3% was applied on the leaves - 6-8 ml per plant, and on the soil - by watering each plant with 30 ml of solution of each dose. Control plants, depending on the type of application, were either sprayed or watered with the equal quantity of tap water. For determination of epiphytic microflora, a weighed averaged sample comprised of 5g of leaves per each variant was placed in a flask with 45ml of sterile distilled water. Soil samples were taken from the rhizosphere zone at a depth of 0 to 5 cm. The sample preparation for epiphytic microflora used the same approach. Petri dishes with nutrient agar (HiMedia, India) were inoculated with 100 µl of 10^{-4} dilution for each variant following the viable plate count standard procedure.

The presented data are averaged values from two year`s experiment (n = 4). Preliminary calculations, log transformation and graphs were done with Excel. The graphs columns presented the mean and error bars - standard deviation. Statistical analysis was done on log-transformed data with SPSS program (IBM, ver. 26). The analysis of skewness and kurtosis of data showed that z-values that exceeded the acceptable range of -1.96, +1.96 (Ghasemi et al., 2012). Additionally, the Levene`s test showed a statistical significance which violated the assumption about the homogeneity of variances and the data normal distribution. Since this assumption, as one of the main prerequisites for conducting the three-way analysis of variance was not met the comparison between variants was done by nonparametric Kruskal-Wallis H-test (Ostertagová et al., 2014).

RESULTS AND DISCUSSIONS

The current study was an integral part of two-year experiment that also assessed the effect of biostimulant Protifert LN 6.5 on several plant characteristics. The details of experimental design and results can be seen in Panayotov & Kartalska (2023). In brief, the results showed that application of Protifert LN 6.5 has a positive effect on the vegetative development of pepper seedlings. The analyzed root and leaves morphological parameters showed the highest values when biostimulant was applied at dose of 2%. The authors considered that the treated pepper seedlings have maintained a harmonious development without excessive growth or significant changes in the weight ratio of plant organs. Such development is a prerequisite for better seedlings adaptation and growth after planting. Furthermore, it was observed that the biostimulant has a positive effect on flower buds since the number of flower buds have increased

in both studied varieties (Panayotov & Kartalska, 2023).
The type of application of biostimulant affected both epiphytic and rhizosphere bacteria. The highest number of epiphytic bacteria was observed after foliar application of Protifert LN 6.5 at dose of 2% with 6.11 (± 0.13) and 5.89 (± 0.09) CFU/g log for variety KK and BR, respectively (Figure 1). The similar trend was observed on the rhizosphere bacteria but to a lower extend. The quantity of rhizosphere bacteria did not exceed 5.59 (± 0.82) and 5.17 (± 0.17) CFU/g for KK and BR variety, respectively.

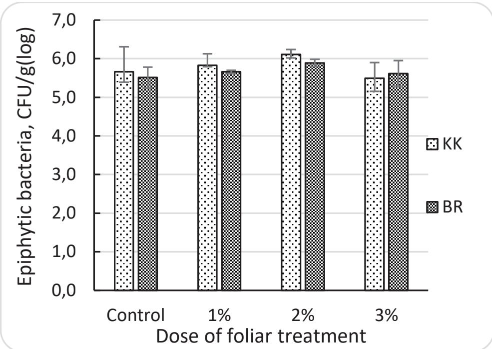

Figure 1. Epiphytic microflora (CFU/g, log) after foliar treatment

The foliar application of biostimulant at a lowest dose of 1% still has some stimulatory effect on the epiphytic bacteria in comparison to the control plants. The effect was more pronounced at dose of 1% for KK variety with value of 5.83 CFU/g (±0.30) than for the BR variety - 5.66 CFU/g (±0.04) with the corresponding control values of 5.66 (±0.65) and 5.51 CFU/g (±0.27), respectively. It appeared that the foliar application of biostimulant also have effect on rhizosphere bacteria. Despite that the differences were relatively small the number of rhizosphere bacteria in both varieties exceeded the control values but the effect was dose dependent since the dose of 2% provided higher values. The effect of foliar application of biostimulant on rhizosphere bacteria at either 1% or 3% for KK variety (5.15 and 5.00 CFU/g) showed that number of bacteria that did not exceed the control value – 5.46 (± 0.88). The same was true for BR variety but only for dose of 1% - 4.89 CFU/g (± 0.19) since the corresponding control was 5.11 CFU/g (± 0.17) and at 3% dose the number of rhizosphere bacteria was higher - 5.19 CFU/g (± 0.11) (Figure 2). It was not expected that the foliar treatment would affect significantly the rhizosphere microflora despite there are some suggestions that due to nutrients` transport in the plants the leaf treatment could affect the composition of root exudates and thus the soil microflora (Tekaya et al., 2021). In the current study, the biostimulant foliar treatment at a dose of 2% showed the higher stimulatory effect on rhizosphere microflora at KK variety (Figure 2).

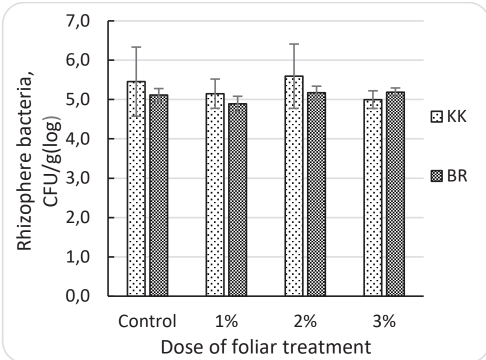

Figure 2. Rhizosphere bacteria (CFU/g, log) after foliar treatment

The soil application has insignificant effect on the number of epiphytic bacteria and the estimated values across the two pepper varieties were very similar. At dose of 2% the two pepper varieties showed the highest values - 5.12 (± 0.09) and 5.11 (± 0.11) CFU/g for KK and BR, respectively. Much alike were the estimated values at 1% and 3% - 4.49 and 4.35 for KK variety and 5.09 and 4.83 for BR variety. None of the values at 1% and 3% exceeded the corresponding controls - 5.05 (± 0.62) and 4.79 CFU/g (± 0.47) for the KK and BR varieties, respectively (Figure 3). The higher value of rhizosphere bacteria after soil application was estimated for KK variety at dose of 2% - 6.27 CFU/g (± 0.27). The same trend was not so noticeable for BR variety which rhizosphere bacteria reached 5.49 CFU/g (± 0.21). The use of amino acids-based biostimulants could improve the assimilation of nutrients and the mineralization of organic matter, which affect the amount of soil microorganisms and their activity (Wadduwage et al., 2024).

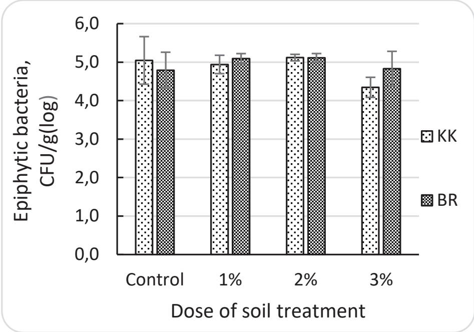

Figure 3. Epiphytic bacteria (CFU/g, log) after soil treatment

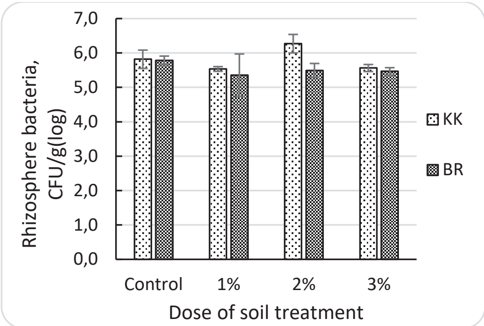

Figure 4. Rhizosphere bacteria (CFU/g, log) after soil treatment

The analysis revealed that application of biostimulant at dose of 1% and 3% did not stimulate the number of rhizosphere microorganisms and their quantity was lower in compareson to the control. For KK variety the two values were very similar - 5.54 and 5.56 at 1% and 3% dose and the corresponding control value was 5.84 CFU/g (± 0.27). The BR variety showed a better correspondence between the doses of application and the number of rhizosphere bacteria since at the dose of 1% the value was 5.35 CFU/g (± 0.62) and at 3% - 5.47 (± 0.11) CFU/g despite that the both was lower than the control 5.78 CFU/g (± 0.13). One possible explanation of the observed trend could be related to the fact that higher doses of amino acids could negatively affect the soil microflora. Kajikawa et al. (2002) reported both stimulatory and inhibitory effect of several amino acids. The microorganism responded differently to single amino acids and to their specific combinations.

In general, when applied at optimal doses organic biostimulants positively affect plant growth and productivity through changes in epiphytic and soil microorganisms (Mahnert et al., 2018). According to Sivojiene et al. (2021) the soil microorganisms are one of the main indicators for assessing stability of a given soil ecosystem and its fertility. The fertilizers can affect the microorganisms and as a result their influence on the soil processes, exchange of substances in the soil-colloid solution and plants mineral absorption (Jacoby et al., 2017, Jannson & Hofmockel, 2018). The use of plant biostimulants can increase the amount of soil microbiota involved in the organic matter decomposition (Sun et al., 2014; Daquiado et al., 2016). According to Hole et al. (2005) the changes in microflora abundance can be used as an indicator for assessment of different organo-minerals applied in the agricultural practice and for determination of potential fertility of soil.

Table 1. The mean rank for effect of dose of biostimulant and type of treatment

Variety/ Treatment	**Factor/ *p* value**	**Rank**			
Foliar treatment	***Dose***[1]	**Control**	**1%**	**2%**	**3%**
KK	p = .219	16.94	15.38	21.69	12
BR	p = .481	15	13.44	20.38	17.19
Type of treatment[2]		E	R		
KK	p < .00	19.94*	13.06		
BR	p < .03	23.91*	9.09		
Soil treatment	***Dose***[1]	**Control**	**1%**	**2%**	**3%**
KK	p = .201	19.13	14.56	20.63	11.69
BR	p = .858	18.69	14.75	16.75	15.81
Type of treatment[2]		E	R		
KK	p < .00	9.16*	23.84		
BR	p < .00	8.5*	24.5		

Legend: [1] - dose, df. 3, n = 8, [2] - type of treatment, df. 1, n = 16, abbr. E - epiphytic, R - rhizosphere bacteria

A Kruskal-Wallis H test did not show a statistically significant difference between the studied doses on the microflora of two pepper varieties (Table 1). However, the variety KK showed higher ranks of microflora both at foliar and soil treatment at dose of 2% but this was true for variety BR only after foliar treatment. The effect of soil treatment on microflora of BR variety at dose of 2% showed a mean rank (16.75) which exceeded the other doses of application but not the control one (18.69). In both pepper varieties, the type of treatment significantly affected the number of cultivable bacteria of the corresponding microflora`s category in an objective and consistent manner.

CONCLUSIONS

The main purpose of application of amino acid-based plant biostimulants is towards increase plant growth and development and alleviation of plant stress. Due to immense role of microorganisms in soil processes and plant productivity the contemporary studies have focused on revealing the effect of biostimulants both on epiphytic or rhizosphere microflora. The results from the current study showed that the application of biostimulant can affect both epiphytic and rhizosphere microflora irrespectively to the type of application. However, the observed effect was dose dependent and the soil application at dose of 3% indicated a slight negative effect on rhizosphere microflora. The observed stimulatory effect on microorganisms could be related either directly to the provided amino acids or to the alteration of some nutrients or bioactive substances. The response of microflora to different doses of applied biostimulant was not statistically significant but despite the relatively similar values, there was a consistent difference between the two pepper varieties. This imply that application of biostimulant should take into account some specifics of crop varieties and their interactions with the microorganisms in order to fulfill its potential as environmental-friendly approach in agricultural practices for vegetable crops.

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THE BEHAVIOR OF SOME MELON GENOTYPES (*CUCUMIS MELO* L.) GROWN ON SANDY SOILS FROM SCDCPN DABULENI

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Abstract

The study was carried out in the period 2022-2023 on the sandy soils of SCDCPN Dăbuleni, characterized by low natural fertility. Six melon genotypes were studied. A great diversity of their morphological and biochemical characteristics was observed. Morphological characteristics such as fruit weight, fruit length and diameter, pulp thickness and fruit shape index were determined for the analyzed fruits. The biochemical characteristics of the fruits, such as total dry matter, soluble dry matter, titratable acidity, carbohydrates, vitamin C and water content, were also analyzed. The best results regarding fruit weight in 2022 (2.48-3.12 kg) were recorded for genotypes: L16, L13, L14 and in 2023 for genotypes L14, L11 and L16 (2.89-3.45kg). The content of soluble dry matter varied in the two years of study between 8.36% at genotype L12 and 9.02% at L14, being a character of the variety, which can also be influenced by environmental conditions. As a result of the study, a great diversity of morphological and biochemical characters was identified, which can be used in the future in the breeding process of existing varieties.

***Key words**: melon**,** morphological characteristics, biochemical characteristics, sandy soils.*

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most important species, from an economic point of view, in the Cucurbitaceae family. *Cucumis melo* (L.) is native to Africa (Nasrabadi et al., 2012; Kerge and Grum, 2000; Robinson and Decker-Walters, 1999). Currently, both wild and cultivated forms of melons are cultivated worldwide (Nasrabadi et al., 2012; Pitrat, 1991).
The world production of yellow melon is over 31 million tons being obtained predominantly by the mediterranean countries and East Asia, of which China provides 51%, followed by Iran and Turkey with a percentage of 5 and 6%, respectively of the world production (Maleki et al., 2018; Pavan et al., 2017; FAO, 2016).
Cucumis melo L. is cultivated because of its juicy and aromatic fruits, which are consumed fresh at physiological maturity, they are rich in vitamins (C, B1, B2, B6), carotene, mineral salts (Ca, P, K, Fe), proteins and carbohydrates (Frățuțu et al., 2023; Drăghici et al., 2018; Ciuciuc, 2003; Cabello et al., 2009).
The information regarding the genetic variability of the *Cucumis melo* L. species is useful to breeders in the process of selecting valuable parental genotypes, which can be used in breeding processes in order to obtain new cultivars (Naroui Rad et al., 2017; Naroui Rad et al., 2015; Naroui Rad et al., 2014).
This main objective of this study is the analysis of the local melon germplasm and the morphological characterization of the cultivated genotypes, with the aim of using them in future breeding programs.

MATERIALS AND METHODS

The experiment was established on the sandy soils of the research field of SCDCPN Dăbuleni, at its location using the randomized block method.
The plant material used to establish the experience in the two years of study 2022-2023 was represented by six melon genotypes L11, L12, L13, L14, L15 and L16.
Some physical properties of the fruits were determined, such as the height and diameter of the fruits (cm), the diameter of the fruit cavity (cm), the shape index, the weight of the fruit (kg) and the thickness of the pulp (cm), according to the methodology described by Ionică (2014).
The chemical properties of the fruits were also determined according to the methodology described by Ionică (2014). Soluble dry matter (SUS) was determined by the refractometric

method, the results being expressed as percentages (%). To determine the content of total dry matter (SUT), the gravimetric method was applied based on the removal of water by evapotranspiration from the average analytical sample used, keeping it in the oven at a temperature between 85-105°C. The results were expressed in percentage of total dry matter (%). The determination of titratable acidity (TA) was carried out using the method described by Ionică (2014), the results were expressed in grams of malic acid/100 g of fresh substance.

To determine the vitamin C content, the iodometric method described by Croitoru (2021) was applied, which is based on the oxidation of ascorbic acid with excess iodine, the results being expressed in mg of ascorbic acid.

Carbohydrates were determined according to the Fehling Soxhelt method described by Croitoru (2021), the results being expressed in percentages.

Statistical analysis

The obtained data were processed statistically, using the statistical analysis program (Stat Point Technologies, Warrenton, VA, USA). Relationships between fruit physical characteristics were quantified using correlations.

RESULTS AND DISCUSSIONS

Abiotic factors including temperature, light, water and nutrients can affect the development of physiological processes and fruit size (Katsumi et al., 1999). Following the soil analyses according to the data in Table 1, a carbon content of the soil was recorded, at the depth of 0-20 cm, in the amount of 0.56% and at 20-40 cm a content of 0.37%, and a value of 0.96% humus at the depth of 0-20 cm and the pH recorded values between 7.10 and 7.12.

Table 1. Analysis of sandy soil composition from experimental field of RDSPCS Dăbuleni

The variant	The depth (cm)	Nt (%)	Extractable phosphorus (ppm)	Exchangeable potassium (ppm)	Carbon organic (%)	Humus (%)	pH
Experimental field	0-20	0.07	20.8	76.8	0.56	0.96	7.12
	20-40	0.03	36.28	44.8	0.37	0.63	7.10
Fertility status		LOW	MEDIUM	LOW	LOW	LOW	NEUTRAL

Melon loves heat, needing an optimal soil temperature of 20-25°C, being a species that prefers light soils with a pH between 6 and 7 (Toma et al., 2011). Table 2 presents the results obtained in 2022 regarding the main biometric characteristics of the fruits of the analyzed melon genotypes.

Table 2. Biometric characteristics of fruits from melon genotypes studied in 2022

Genotype	Statistical analysis	Fruit weight (kg)	Fruit height (cm)	Fruit diameter (cm)	Pulp thickness (cm)	IF
L11	Mean ± SD	2.17 ± 0.65	26.38 ± 1.92	13.80 ± 1.54	3.10 ± 0.68	1.92 ± 0.08
	Variation limits	1.49 / 2.92	24.20 / 28.50	12.00 / 15.40	2.00 / 3.70	1.84 / 2.02
	CV %	29.8	7.27	11.19	21.88	4.25
L12	Mean ± SD	1.72 ± 0.42	21.18 ± 3.93	14.68 ± 0.35	3.57 ±0.61	1.44 ± 0.25
	Variation limits	1.43/2.47	17.20 / 26.90	14.30 / 15.10	2.53 / 4.00	1.20 / 1.79
	CV %	24.61	18.53	2.37	17.11	17.34
L13	Mean ± SD	2.68 ± 0.33	26.13 ± 2.21	15.30 ± 1.42	3.53 ± 0.44	1.72 ± 0.19
	Variation limits	2.30 / 3.13	24.20 / 28.57	14.20 / 17.30	2.80 / 4.00	1.50 / 2.01
	CV %	12.31	8.46	9.28	12.58	10.78
L14	Mean ± SD	3.12 ± 0.97	24.62 ± 3.21	17.86 ± 2.50	4.04 ± 0.80	1.39 ± 0.15
	Variation limits	2.07 / 4.69	19.90 / 28.60	15.50 / 21.50	3.30 / 5.20	1.24 / 1.65
	CV %	31.15	13.02	14.02	19.85	11.07
L15	Mean ± SD	1.95 ± 0.87	23.62 ± 2.81	13.64 ± 2.80	3.42 ± 0.64	1.76 ± 0.23
	Variation limits	1.10 / 3.37	21.10 / 28.10	10.51 / 18.00	2.50/4.00	1.56 / 2.01
	CV %	44.45	11.91	20.54	18.56	12.86
L16	Mean ± SD	2.48 ± 0.83	26.02 ± 6.18	15.44 ± 1.74	3.86 ± 0.42	1.70 ± 0.41
	Variation limits	1.51 / 3.78	17.90 / 34.00	13.20 / 17.50	3.50 / 4.40	1.23 / 2.08
	CV %	33.62	23.73	11.24	10.78	24.39

IF = Shape index; CV = coefficient of variability; SD = standard deviation.

In the year 2022, the melon fruits showed an average height that varied between 26.38 cm for the genotype L11 and 21.18 cm for the fruits of the genotype L12. The diameter of the fruits presented an average value between 17.86 cm for the L14 genotype and 13.64 cm for the L15 genotype. The shape index showed average values that varied between 1.92 for genotype L11 and 1.39 for genotype L14, which indicates an elongated shape of the melons analysed. Regarding the length of the fruits and their diameter, in the literature, Rad et al. (2017), reported lower values than those obtained in this study, namely 20.00 cm with variation limits between 11.00 and 33.00 cm for the length of the fruits; and a diameter of 12.03 cm with variation limits between 6.5 and 15.8 cm.

Figure 1. Fruit from L12 genotype (original)

An important characteristic analysed in melon fruits is their weight, which recorded in 2022 the highest value of 3.12 kg for the L14 genotype, with variation limits between 2.07 and 4.69 kg, and the lowest weight of 1.72 kg in the L12 genotype with variation limits between 1.43 and 2.47 kg. For the genotypes studied in 2022, the pulp thickness recorded the highest value of 4.04 cm for the fruits of the L14 genotype with variation limits between 3.30 and 5.20 cm, and the lowest for the L11 genotype, respectively 3.10 cm, with variation limits between 2.00 and 3.70 cm. The results obtained are in accordance with the specialized literature, but higher than those reported by Rad et al. (2017), for the studied genotypes respectively an average fruit weight of 1.52 kg with variation limits between 0.495 and 3.128 kg. Regarding pulp thickness, Rad et al. (2017), reported a mean value of 7.04 cm with ranges of variation between 5.00 and 8.75 cm, much higher than those reported in the present study. Regarding the coefficient of variability, according to the data in Table 2, it can be seen that in 2022 its highest values were recorded for fruit weight, which recorded values between 12.31% (L13) and 44.45% to the L15 genotype. It can thus be noted that in 2022 the genotype L14 stood out with the highest average values obtained for fruit weight and pulp thickness. Table 3 presents the results regarding the biometric characteristics of the melon fruits analysed in 2023.

Table 3. Biometric characteristics of fruits from melon genotypes studied in 2023

Genotype	Statistical analysis	Fruit weight (kg)	Fruit height (cm)	Fruit diameter (cm)	Pulp thickness (cm)	IF
L11	Mean ± SD	2.92 ± 0.98	24.76 ± 4.12	15.66 ± 1.60	3.94 ± 0.95	1.59 ± 0.25
	Variation limits	1.73/4.18	19.00 / 30.00	13.30 / 17.50	2.80 / 5.40	1.27 / 1.84
	CV %	33.46	16.63	10.2	24.19	15.46
L12	Mean ± SD	2.77 ± 0.75	26.6 ± 5.55	16.24 ± 2.19	3 ± 0.61	1.63 ± 0.23
	Variation limits	1.44 / 3.24	20.00 / 35.00	13.00 / 19.00	2.00 / 3.50	1.47 / 2.03
	CV %	27.04	20.86	13.47	20.41	14.14
L13	Mean ± SD	1.74 ± 0.44	20.42 ± 1.85	14.44 ± 1.32	3.16 ± 0.78	1.42 ± 0.11
	Variation limits	1.288 / 2.248	18.00 / 23.00	13.00 / 16.30	2.00 / 4.00	1.29 / 1.54
	CV %	25.28	9.07	9.12	24.57	7.95
L14	Mean ± SD	2.89 ± 0.46	23.6 ± 3.07	17.52 ± 1.79	3.94 ± 0.36	1.35 ± 0.14
	Variation limits	2.53 / 3.65	20.50 / 27.50	15.70 / 20.00	3.40 / 4.40	1.15 / 1.49
	CV%	15.75	13.01	10.19	9.08	10.66
L15	Mean ± SD	1.99 ± 0.37	16.88 ± 1.68	16.52 ±2.09	4.02 ± 0.18	1.04 ± 0.17
	Variation limits	1.39 / 2.39	15.00 / 19.00	13.00 /18.50	3.80 / 4.30	0.86 / 1.27
	CV %	18.56	9.96	12.66	4.45	16.27
L16	Mean ± SD	3.45 ± 0.63	25.44 ± 1.56	16.92 ± 0.50	4.2 ± 0.24	1.5 ± 0.10
	Variation limits	2.88 / 4.48	24.00 / 28.00	16.20 / 17.50	4.00 / 4.60	1.42 / 1.68
	CV %	18.25	6.14	2.94	5.83	6.57

IF = Shape index; CV = coefficient of variability; SD = standard deviation.

The highest value of fruit weight was recorded for genotype L16, respectively 3.45 kg, with variation limits between 2.88 and 4.48 kg, in contrast to genotype L13 which recorded this year the lowest average weight of fruits respectively 1.74 kg with variation limits between 1.28 and 2.24 kg. Regarding the height of the fruits, the highest value was recorded for the fruits of L12 genotype, respectively 26.6 cm, and the lowest height for the fruits of L15 genotype, respectively 16.88 cm. The diameter of the fruits recorded the highest value of 17.52 cm for genotype L14, with variation limits between 15.70 and 20 cm, and the smallest for the fruits of genotype L13, respectively 14.44 cm with variation limits between 13.00 and 16.30 cm. The thickness of the flesh in the fruits of the analysed melon genotypes in 2023 had values between 3.00 and 4.20 cm in genotypes L12 and L16, respectively. Regarding the shape of the fruits we can see according to the data in the table regarding the shape index as they were elongated in shape with a shape index value between 1.04 and 1.63. Considering the specialized literature, we can specify that the data obtained in the present study are consistent with those reported by Nasrabadi et al. (2012), respectively an average length of the fruits between 22.64 and 48.10 cm, the diameter of the fruits with values between 15.15 and 19.56 cm, and a fruit weight that varied between 2.30 and 4.92 kg. With the help of correlations, the relationships between the biometric characteristics of the fruits were examined, the results being shown in Table 4.

Analysing the data in Table 4, we can see that in both years of the study, positive correlations were calculated between the height of the fruit and the weight of the fruit (r = 0.69, respectively r = 0.74), and between fruit diameter and weight (r = 0.81, respectively r = 0.61).

Figure 2. Fruit from L15 genotype (original)

A positive correlation was also calculated between the diameter and thickness of the fruit pulp (r = 0.72 and r = 0.51, respectively).

Tabel 4. Correlations between the fruit characteristics of the melon genotypes studied in the two years 2022-2023

Year	Fruit weight (kg)		Fruit height (cm)		Fruit diameter (cm)		Pulp thickness (cm)		IF
	2022	2023	2022	2023	2022	2023	2022	2023	2022
Fruit weight (kg)	1	1							
Fruit height (cm)	0.69	0.74	1	1					
Fruit diameter (cm)	0.81	0.61	0.33	0.37	1	1			
Pulp thickness (cm)	0.51	0.58	0.04	0.26	0.72	0.51	1	1	
IF	0.53	0.41	0.17	0.83	0.63	-0.21	0.36	-0.06	1

IF = Shape index

The data obtained from the correlations in the present study are consistent with those reported in the literature by Rad et al. (2017), who reported positive correlations between fruit weight and length (r = 0.77), between weight and fruit diameter (r = 0.63) and between fruit pulp weight and thickness (r = 0.32). The taste of fruits is the main determinant of their quality. The amount of sugar influences the sweetness and economic value of melon fruits (Kolayli et al., 2010). In order to highlight the nutritional and taste qualities of melons, chemical properties shown in Table 5 were selected and evaluated. According to the recorded data, we can see that the soluble dry matter recorded an average value between 9.06% for the L14 genotype and 8.36% for the L12 genotype in the two analysed years. The total dry matter content showed values that varied between 7.05% (L15) and 9.64% at L12. Regarding the vitamin C content, it varied in the analysed genotypes, between 16.26 mg/100 g FW in L15, and 29.04 mg/100 g FW in the L13 genotype.

Tabel 5. Chemical properties of fruits from melon genotypes studied during 2022-2023

Genotype	SUS %	SUT %	TA g ac malic/100 g substance	Vit. C mg	Glucide %	Water %
L11	8.80	7.93	0.23	22.00	6.36	92.07
L12	8.36	9.64	0.26	27.72	7.78	90.37
L13	8.96	7.30	0.21	29.04	6.19	92.71
L14	9.06	9.36	0.24	20.68	7.40	90.65
L15	8.75	7.05	0.18	16.26	7.62	92.95
L16	8.76	9.16	0.22	18.92	7.40	90.85

* SUT = total dry substance; SUS = total soluble substance; TA = titrable acidity; Vit. = vitamin

Titratable acidity and carbohydrates had values that varied between 0.18 g (L15) and 0.26 g (L12) malic acid/100 g FW, respectively 6.19% and 7.78% carbohydrates; and the water content between 90.37% (L12) and 92.95% (L15).

The variability of the sugar content of the fruits of melon genotypes was also reported in the literature by Rad et al. (2017) and Kolayli et al. (2010), who mentioned a sugar content that oscillated between 2.25% and 8.75% and 6.90% and 9.60% Brix respectively; the values reported in the present study being higher than those reported by Rad et al. (2017) and consistent with those reported by Kolayli et al. (2010). Kolayli et al. (2010) reported at the analysed melon genotypes a content of ascorbic acid with values between 5.38 and 22.47 mg/100 g FW.

Figure 3. Determination of biochemical characteristics (original)

CONCLUSIONS

Following the research carried out in the two years of study, the genotypes L14 and L16 were noted. Genotype L14 stood out in both years of study in terms of fruit diameter (17.86 cm and 17.52 cm, respectively) and soluble dry matter content of 9.06% Brix. Genotype L16 stood out in 2023 due to the weight and thickness of the fruit pulp, respectively 3.45 kg and 4.20 cm.

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PHYSICO-CHEMICAL CHARACTERISTICS OF PUMPKIN FRUITS (*CUCURBITA MAXIMA* DUCH.) CULTIVATED ON SANDY SOILS AT SCDCPN DĂBULENI

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Abstract

The edible pumpkin is grown for its fruits, which can be consumed riped, boiled, or candied. In Romania, the pumpkin culture has recently started attracting the growers attention. In this context, at the Dăbuleni research station, six pumpkins (three cultivars `Marele alb`, `Coroana Prințului`, `Tudor`, and three genotypes D19, P1, P2) were studied to analyze their nutritional potential. Observations regarding the physical characteristics of the fruits (height, diameter, and weight of the fruit, diameter of the fruit cavity, shape index, and pulp thickness) were analyzed, as well as observations regarding the food quality (soluble dry matter, total dry matter, malic acid, carbohydrates, vitamin C). The thickness of the fruit pulp varied between 3.30 cm for `Tudor` and 4.50 cm for the `Coroana Prințului` cultivars, and the fruit weight varied between 3.16 kg (P2) and 8.20 kg for the `Marele Alb`. A content in soluble dry matter of more than 11% was presented at the `Marele alb`, P2, and D19.

Key words: *pumpkin, morphometric characteristics, biochemical characteristics, sandy soils.*

INTRODUCTION

Cucurbita maxima is an annual species of the *Cucurbita* genus from the *Cucurbitaceae* family, which grows as a bush or creeps on the ground, its fruits being an important, but still underestimated, raw material, which can be processed in the form of natural juices, creams, jams, marmalades, or consumed as dry or candied snacks (Niewczas et al., 2014). Pumpkin was included in the category of useful vegetables due to its rich content of phenols, flavonoids, vitamins (carotene, vitamin A, α-tocopherol and vitamin C), amino acids and carbohydrates, thus showing great importance for human nutrition and economy (Zhang et al., 2000; 2002; Wang et al., 2002; Dinu et al., 2016; Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997; Kulkarni and Joshi, 2013; Ping et al., 2002).
The numerical growth of the population together with the unpredictable climate changes and the decrease in soil fertility, have forced the exploration of alternative underutilized flora, while there is also a great opportunity for economic growth in the agricultural system by introducing underutilized products to the market (Sharma & Ramana Rao, 2013). Because pumpkin cultivars differ in terms of nutritional values, producers and researchers are searching for the most valuable and nutritionally appropriate genotypes (Dinu et al., 2016). There are few scientific studies on the physiological, chemical, physicochemical, nutritional, functional and technological characteristics of pumpkin. Most researchers agree with the need for a greater number of investigations, in order to better capitalize on the pumpkin culture (Ahmad and Khan, 2019).

MATERIALS AND METHODS

The experiment was located on the sandy soils of the Dăbuleni RDSPCS (Research-Development Station for Plant Culture on Sands) from the south of Oltenia, and for its establishment, the randomized block method was used.
The plant material used to establish the experience in the two years of study 2021-2022 was represented by six pumpkin cultivars 'Marele alb', 'Coroana prințului', 'Tudor', D19, P1 and P2.
The physical properties of the fruits, determined in the two years, were represented by the height and diameter of the fruits (cm),

the diameter of the fruit cavity (cm), the shape index, fruit weight (kg) and pulp thickness (cm), according to the methodology described by Ionică (2014). The chemical properties of pumpkin fruits were determined according to the methodology described by Croitoru (2021). Soluble dry matter (SUS) was determined by the refractometric method, the results being expressed as percentages (%). To determine the content of total dry matter (SUT), the gravimetric method was applied based on the removal of water by evapotranspiration from the average analytical sample used, keeping it in the oven at a temperature between 85-105°C. The results were expressed as a percentage of total dry matter (%).
The determination of titratable acidity (TA) was carried out using the method described by Ionică (2014), the results were expressed in grams of malic acid/100 g of fresh substance. For determination of the vitamin C content, the iodometric method described by Croitoru (2021) was applied, which is based on the oxidation of ascorbic acid with excess iodine, and the results were expressed in mg of ascorbic acid.
Carbohydrates were determined according to the Fehling Soxhelt method described by Croitoru (2021), the result being expressed in percentages. The pH of pumpkin fruits was determined according to the method described by Rangana (1977), mentioned by Sharma and Rao (2013).
The obtained data were processed statistically, using the statistical analysis program (StatPoint Technologies, Warrenton, VA, USA). Relationships between fruit physical characteristics were quantified using correlations.

RESULTS AND DISCUSSIONS

Analyzing the results from Table 1, it can be seen that there are differences between the two years of study, both from a thermal and hydrological point of view. These differences influenced the morphological characteristics and biochemical composition of pumpkin fruits to a certain extent. During the two years of the study, the temperatures recorded during the May-August vegetation period were higher than the multiannual average. From the point of view of the amount of precipitation in 2021, the months of May and June recorded amounts of 55 mm and 53 mm, respectively, much lower than those of April and June 2022 when precipitation was recorded in the amount of 73.6 mm and 67.4 mm, respectively. In the two years analyzed, it can be observed that the month of July was the driest, with the amount of precipitation being between 15 mm (2022) and 16.8 mm (2021).

Table 1. Average air temperature and amount of precipitation during the pumpkin growing season

Month	Temperature (°C)			Rainfall (mm)		
	2021	2022	1956-2022*	2021	2022	1956-2022*
April	9.72	11.70	11.88	30.6	73.6	46.97
May	17.6	18.3	16.95	55	38.4	62.39
June	21.7	22.9	21.55	53	67.4	69.83
July	25.7	25.2	23.29	16.8	15	54
August	24.5	25.1	22.66	9.00	49.4	36.76

Table 2 shows the results obtained in 2021 regarding the main biometric characteristics of pumpkin fruits. In this year, the pumpkin fruits showed an average height that varied between 31.10 cm for the 'Tudor' cultivar and 9.30 cm for the fruits of the D19 genotype. The diameter of the fruits showed an average value between 26.60 cm for the 'Marele alb' and 11.50 cm for the 'Tudor' cultivar. Regarding the diameter of the cavity of the analyzed fruits, during 2021, recorded values varied between 22.50 cm for the fruits of 'Marele alb' cultivar and 6.10 cm for the fruits of the 'Coroana prințului'. The shape index presented average values that varied between 0.43 cm for the D19 genotype and 2.76 cm for the 'Tudor'.
An important characteristic analyzed for pumpkin fruits is their weight, which recorded in 2021 the highest prediction of 8.20 kg for the 'Marele alb' cultivar, with variation limits between 5.00 kg and 11.30 kg, and the lowest weight of 3.16 kg in the P2 genotype with variation limits between 2.40 and 4.40 kg. Ahmed (2017) reported for the analyzed cultivars an average fruit weight that varied between 1.41 kg and 5.78 kg, lower than those recorded in the present study.
The pulp thickness of the analyzed pumpkin fruits varied between 4.50 cm in the 'Coroana prințului' cultivar and 3.52 cm in the fruits of the P2 genotype. In the specialized literature, Niewczas et al. (2014) reported for the cultivars analyzed values that confirm the results

obtained in the present study, respectively a height of the fruits between 10.06 cm and 29.90 cm, the diameter of the fruits between 17.20 cm and 28.20 cm and a pulp thickness between 3.00 cm and 4.60 cm. Analyzing the data from Table 2, it appears that in 2021 the 'Marele alb' cultivar stood out with the highest average values obtained for fruit diameter and fruit weight, and for the thickness of the flesh of the pumpkin fruits, the 'Coroana prințului' cultivar stood out.

Table 2. Biometric characteristics of fruits from pumpkin fruits studied in 2021

Cultivar/ Genotype	Statistical analysis	Fruit height (cm)	Fruit diameter (cm)	Cavity diameter (cm)	Shape index (cm)	Fruit weight (kg)	Pulp thickness (cm)
'Marele alb'	Mean ± SD	27 .00 ± 2.92	26.60 ± 4.28	22.50 ± 3.99	1.02 ± 0.10	8.20 ± 2.76	4.30 ± 0.67
	Variation limits	23.00 – 30.00	23.00 – 33.00	19.00 – 28.00	0.88 – 1.17	5.00 – 11.30	3.50 – 5.00
	CV%	10.80	16.08	17.91	10.06	33.69	15.6
'Coroana prințului'	Mean ± SD	22.50 ± 1.12	10.60 ± 1.08	6.10 ± 1.02	2.15 ± 0.29	3.50 ± 0.40	4.50 ± 0.61
	Variation limits	21.00 – 24.00	9.00 – 12.00	4.50 – 7.00	1.75 – 2.56	3.00 – 4.00	3.50 – 5.00
	CV%	4.97	10.23	16.8	13.36	11.43	13.61
'Tudor'	Mean ± SD	31.10 ± 2.90	11.50 ± 2.24	7.90 ± 2.07	2.76 ± 0.42	6.56 ± 1.27	3.60 ± 0.26
	Variation limits	26.50 – 34.00	9.00 – 15.00	5.50 – 11.00	2.10 – 3.24	4.40 – 7.60	3.30 – 4.00
	CV%	9.33	19.44	26.23	15.13	19.36	7.35
D19	Mean ± SD	9.30 ± 0.76	21.80 ± 1.04	18.20 ± 1.04	0.43 ± 0.03	3.28 ± 0.26	3.60 ± 0.55
	Variation limits	8.50 – 10.00	21.00 – 23.50	17.50 – 20.00	0.39 – 0.48	2.50 – 4.00	3.00 – 4.50
	CV%	8.15	4.76	5.7	8.1	17.96	15.21
P1	Mean ± SD	26.61 ± 2.52	21.82 ± 3.55	17.78 ± 2.95	1.24 ± 0.18	7.74 ± 2.15	4.04 ± 0.74
	Variation limits	23.00 – 29.50	19.50 – 28.00	15.80 – 23.00	1.00 – 1.51	5.00 – 10.80	3.00 – 5.00
	CV%	9.48	16.29	16.59	14.93	27.8	18.9
P2	Mean ± SD	18.00 ± 5.18	16.60 ± 4.38	13.08 ± 4.40	1.20 ± 0.62	3.16 ± 0.89	3.52 ± 0.89
	Variation limits	10.50 – 25.00	12.00 – 21.50	8.30 – 17.90	0.54 – 2.08	2.40 – 4.40	3.00 – 3.80
	CV%	28.8	26.38	33.68	23.2	28.18	8.85

SD = standard deviation; CV = coefficient of variability

Table 3. Biometric characteristics of pumpkin fruits studied in 2022

Cultivar / Genotype	Statistical analysis	Fruit height (cm)	Fruit diameter (cm)	Cavity diameter (cm)	Shape index (cm)	Fruit weight (kg)	Pulp thickness (cm)
'Marele alb'	Mean ± SD	30.4 ± 5.09	24.48 ± 2.33	16.31 ± 2.32	1.25 ± 0.25	8.16 ± 2.05	4.34 ± 0.57
	Variation limits	25.70 – 38.00	21.60 -27.20	14.00 – 19.50	0.94 – 1.46	6.19 – 11.58	3.50 – 5.00
	CV%	16.75	9.54	14.26	19.68	25.14	13.1
'Coroana prințului'	Mean ± SD	10.80 ± 1.52	25.44 ± 2.40	16.70 ± 2.25	0.43 ± 0.05	4.78 ± 1.41	4.60 ± 1.29
	Variation limits	9.00 – 13.00	22.40 – 28.50	13.50 – 19.00	0.35 – 0.48	2.94 – 6.42	3.00 – 6.50
	CV%	14.12	9.45	13.45	11.91	29.4	28.14
'Tudor'	Mean ± SD	13.60 ± 1.50	28.84 ± 2.96	21.24 – 3.37	0.48 ± 0.08	6.57 ± 1.15	4.34 ± 0.21
	Variation limits	11.60 - 15.60	25.60 - 32.20	18.00 – 25.00	0.36 – 0.59	4.90 – 7.74	4.10 – 4.60
	CV%	11	10.25	15.88	17.65	17.55	4.78
D19	Mean ± SD	8.80 ± 0.63	23.30 ± 2.78	15.82 ± 2.45	0.38 ± 0.03	3.78 ± 1.47	3.40 ± 0.96
	Variation limits	8.00 – 9.70	21.00 – 28.00	12.50 – 19.30	0.34 – 0.42	2.86 – 6.33	2.50 – 5.00
	CV%	7.14	11.93	15.51	8.98	38.99	28.29
P1	Mean ± SD	18.48 ± 3.16	25.96 ± 2.51	18.56 ± 2.55	0.71 ± 0.11	5.84 ± 1.39	3.58 ± 0.56
	Variation limits	16.00 – 23.02	22.50 – 29.00	16.30 – 22.50	0.59 – 0.84	3.94 – 7.49	3.00 – 4.20
	CV%	17.09	9.66	13.76	15.47	23.79	15.6
P2	Mean ± SD	20.74 ± 6.96	29.60 ± 2.06	21.50 ± 3.06	0.71 ± 0.25	8.77 ± 1.86	3.96 ± 0.99
	Variation limits	15.50 – 31.00	27.50 – 33.00	17.50 – 25.50	0.47 – 1.05	6.69 – 10.79	2.50 – 5.00
	CV%	33.57	6.97	1423	34.92	21.24	24.91

SD = standard deviation; CV = coefficient of variability

In the year 2022, it can be observed, analyzing the results in Table 3, that the highest average values were obtained by the 'Marele alb' cultivar, for the height and shape index of the fruits respectively 30.40 cm and an index of 1.25. For the pulp thickness, the 'Coroana prințului' cultivar stood out with an average value of 4.60 cm, and the genotype P2 for the highest average values of the respective fruit diameter of 29.60 cm and an average fruit weight of 8.77 kg. The lowest average values recorded in 2022 for all analyzed biometric characteristics were quantified by the fruits of D19 genotype.

The data obtained in 2022, in the present study, are consistent with those reported in the literature by Ferriol et al. (2004), respectively an average weight between 1.5 kg and 18.7 kg, a diameter with values between 13.6 cm and 43.5 cm and a thickness of the pulp that varied between 1.6 cm and 5.5 cm. Ahmed et al., (2017) reported a diameter of the fruit cavity between14.04 cm and 25.44 cm for the studied cultivars. The relationships between the biometric characteristics of the fruits were examined with the help of correlations and the results are presented in Table 4. In the year 2021, high positive correlations were calculated between the height of the fruit and the shape index (r = 0.69), and between height and weight fruit (r = 0.72). A positive correlation was also calculated between fruit diameter and weight (r = 0.50) and between fruit cavity weight and diameter (r = 0.47).

In 2022 high positive correlations were calculated between height and fruit shape index (r = 0.97), fruit height and weight (r = 0.71), fruit diameter and weight (r = 0.69), and between the weight of the fruit and the diameter of its cavity (r = 0.56).

Analyzing the correlations between the biometric characteristics regarding the thickness of the pulp, it was observed that it is influenced by the weight of the fruit, recording correlation values between r = 0.35 in 2021 and r = 0.32 in 2022.

Table 4. Correlations between the characteristics of pumpkin fruits analyzed in the two years (2021-2022)

Characteristics	Fruit height (cm)		Fruit diameter (cm)		Cavity diameter (cm)		Shape index (cm)		Fruit weight (kg)		Pulp thickness (cm)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Fruit height (cm)	1.00	1.00										
Fruit diameter (cm)	-0.16	0.05	1.00	1.00								
Cavity diameter (cm)	-0.18	0.01	1.00	0.91	1.00	1.00						
Shape index (cm)	0.69	0.97	-0.79	-0.18	-0.81	-0.20	1.00	1.00				
Fruit weight (kg)	0.72	0.71	0.50	0.69	0.47	0.56	0.10	0.53	1.00	1.00		
Pulp thickness (cm)	0.24	0.13	0.18	0.21	0.08	-0.07	0.02	0.09	0.35	0.32	1.00	1.00

Pumpkin genotypes differ in color, size and shape, showing a hard rind with thick, edible flesh around the seed cavity (Hosen et al., 2021). Morphological characterization is essential in elucidating the genetic relationships between different groups of *Cucurbita* species (Barzegar et al., 2013). Fruit shape morphology is one of the most diverse traits and depends on geographic origin (i.e., adaptation to environmental factors), cultural traditions, culinary attributes, and market characteristics and requirements (Staub et al., 2000).

According to the data in Table 5, the pumpkin genotypes studied at Dăbuleni RDSPCS in the two years, presented a round fruit shape, slightly elongated at 'Marele alb' cv. and P1, elongated at 'Coroana prințului' cv., 'Tudor' cv. and P2, and at D19 a flattened shape. Regarding the size of the fruits, they presented large (> 4 kg) and very large (> 6 kg) fruits, this classification being made according to the scale used by Abdein in 2018.

Table 5. Classification of pumpkin fruits in the genotypes studied, according to shape, size, peel and pulp color

Cultivar/ Genotype	The shape of fruits	The size of fruits*	The skin color of the fruit	The color of the fruit pulp
'Marele alb'	Round, slightly elongated	Extra large	White-grey	Yellow
'Coroana prințului'	Elongate	Large	Dark green	Orange
'Tudor'	Elongate	Large	Light greens	Orange
D19	Flattened	Medium	White-reddish	Orange
P1	Round, slightly elongated	Extra large	Dark green	Yellow
P2	Elongate	Large	Light green	Orange

*small (< 2 kg), medium (2-4 kg), large (> 4 kg), extra-large (> 6 kg)

The color of the skin varied from shades of white-gray to 'Marele alb' cv., red-white in D19, light green in 'Tudor' cv. and dark green in the rest of the genotypes. As for the color of the fruit pulp, it varied between yellow ('Marele alb' and P1) and orange in the rest of the analyzed genotypes.

The sensory qualities of the products, which determine their value from the point of view of consumers, are represented by the content of sugar, organic acids, phenolic compounds, volatile substances, etc. In this sense, the main chemical properties of pumpkin fruits were analyzed in 2022, the results being presented in Table 6. Soluble dry matter (SUS, %) varied between 12.60% in genotype D19 followed by P2 with 12.00%, and 6.40% in genotype P1. Total dry matter content (SUT, %) showed values between 17.40% at P2 and 10.00% at D19. The highest value of titratable acidity (TA) of 0.26 g malic acid/100 g substance was recorded in 'Tudor' cv. and the lowest in D19 genotype, respectively 0.12 g malic acid/100 g substance. Vitamin C had values between 13.20 mg/100 g fresh substance in D19 genotype and 23.70 mg/100 g fresh substance in P1 genotype.

Regarding the carbohydrate content, it varied between 5.51% in P1 and 17.21% in P2 genotype. The pH of the pumpkin fruits recorded for the analyzed genotypes, had values that varied between 5.90 for P1 and 6.20 for D19 genotype. The results obtained are in accordance with the specialized literature, but lower than those reported by Sharma and Rao (2013), respectively a pH with values between 5.66 and 6.72, a titratable acidity with a value of 0.38% and a vitamin C content that varied between 4.3 mg and 15 mg/100 g of fresh substance. Regarding the content of soluble dry matter, the results obtained are higher than those reported by Javaherashti et al. (2012) respectively a content with values between 6.83% and 8.47%, and consistent with those reported by Dinu et al. (2016), respectively values between 12.60% and 5.51%. Regarding the total content of dry matter, Dinu et al. (2016) reported values between 7.53% and 12.42% lower than those recorded in the studied cultivars. Zinash et al. (2013) recorded in the analyzed pumpkin fruits a total content of dry matter with values between 6.00% and 11.00%, which confirms the results obtained in the present study. Selvi et al. (2012) reported for the cultivars analyzed a carbohydrate content with average values between 0.73 g and 2.95 g/100 g substance. The modification of the pH of the pulp of pumpkin fruits influences the activity of enzymes during the ripening period, and the antioxidant system, subsequently affecting their sensory quality (McCollum et al., 1988).

Table 6. Chemical properties of pumpkin fruits analyzed in 2022

Cultivar/ Genotype	SUS (%)	SUT (%)	TA (g malic acid/ 100 g subst)	Vit C (mg/ 100 g)	Carbohy drates (%)	pH
'Marele Alb'	11.0	10.3	0.16	16.70	8.43	5.98
'Coroana prinţului'	9.8	15.0	0.18	21.20	10.84	6.05
'Tudor'	8.0	14.1	0.26	18.40	9.46	6.08
D 19	12.6	10.0	0.12	13.20	6.88	6.20
P1	6.4	16.0	0.16	23.70	5.51	5.90
P2	12.0	17.4	0.16	20.20	17.21	6.11

*SUT = total dry substance; SUS = total soluble substance; TA = titratable acidity; Vit C = vitamin C.

CONCLUSIONS

Following the study, the 'Marele alb' cultivar stood out for the height, diameter, weight and diameter of the fruit cavity, and the 'Coroana prinţului' cultivar stood out for the pulp thickness.

The D19 genotype is also notable for its very sweet taste with a content of 12.60% dry matter.

The present study provides information about less used agricultural products, allowing us to make better use of this category of nutritionally rich products.

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RELATIONSHIP BETWEEN NDVI AND IN SITU DATA IN PEPPER PLANTATIONS ON OPEN FIELD CONDITIONS

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Abstract

Of the spectral vegetation indices, NDVI is one of the most suitable for tracking the dynamics of vegetable crop development. Its values are most accurate in the active phases of pepper growth. Due to the individual nature for the determination of vegetation indices, it is necessary to look for correlation with the analytical measurements of plants. The aim of this paper is to determine the relationship between NDVI and in situ data in pepper plantations, field production in the phases of active plant growth. In situ data collection was carried out in a production pepper plantation, cv. Slonsko uho, grown under field conditions in the village of Katunitsa, Plovdiv region, Bulgaria. Measurements were carried out in two phases - mass flowering (BBCH 59610) and technological maturity (BBCH 73703). Vegetative plants parameters were determined. NDVI was obtained from Sentinel-2 HR multispectral satellite imagery. The relationships between NDVI and in situ data were determined.

Key words: *pepper, productivity, vegetation indices.*

INTRODUCTION

Traditional monitoring of plant development is carried out through field observations and laboratory analyses. These investigate the main characteristics of growth in relation to phenological development, intensity of crop grow and fresh biomass accumulation. These methods are periodical, very intensive and time consuming. Compared to traditional methods, remote sensing is rapid and provides a wide view of the status of the overall production crop (Li et al., 2019). According to Tunca & Köksal (2022), in the last two decades remote sensing has become an important monitoring tool and a main non-instrumental method for diagnostics and forecasting of crop development and productivity.

Many researchers have worked to clarify the practical application issues of vegetation indices, such as Crippen (1990); Friedl et al. (1994); Penuelas et al. (1997); Blackburn (1998); Gobron et al. (2000); Gitelson et al. (2003); Ceccato et al. (2002); Ferencz et al. (2004); Maire et al. (2008); Ahamed et al. (2011); Dang et al. (2011), Omia et al. (2022);

Ji-Hua & Bing-Fang (2008) pointed that the basis of direct observations is the determination of vegetation indices such as NDVI and LAI. The same authors found that the higher the values of these indices, the better the overall condition of the crop. They concluded that satellite data with high frequency and low spatial resolution provide information for quantitative monitoring with vegetation indices playing a key role. To achieve more accurate information, they recommend combining the obtained data with those from GPS and GIS (Arnaudova et al., 2020). NDVI was designed to estimate vegetative mass from reflectance spectral bands from satellite data. According to Sahebjalal & Dashtekian (2013), the generated images and vegetation indices can be used to determine the vegetation changes occurring between two different stages of development. Ozyavuz et al. (2015) determined changes in plants growth and development by tracking changes in NDVI.

Vegetable crops have increasingly been the subject of scientific research in recent years (Na et al., 2017a; 2017b). Pepper takes the fifth place in terms of production compared to all vegetable crops and is a traditional crop for Bulgaria (Shaban, 2014). According to FAO (2010), average yields, worldwide, are relatively low compared to the biological potential of the species. Therefore, there is a clear need to use innovative approaches to monitoring vegetable plants in order to design and conduct agronomic practices. Na et al.

(2018) estimated the growth of red pepper by determining the vegetation index NDVI at different stages of plant development. Tunca & Köksal (2022) estimated the yield of pepper by time series of several vegetation indices, incl. NDVI. Karaca et al. (2023) compared traditional chlorophyll and fluorescence measurement methods and NDVI in predicting productivity and yield in pepper and melon. The available scientific information is limited for the development of practical methods for remote sensing of vegetable crops using vegetation indices.
The main aim of this paper is to determine the relationship between NDVI and *in situ* data in pepper plantations, field production in the stages of plant growth.

MATERIALS AND METHODS

Study area

The open field located in the South-central region of Bulgaria in the village of Katunitsa, municipality Sadovo, Plovdiv district (Figure 1 a, b).

a) L 42° 6'49.69"N B 24°52'38.77"E (2022)

b) 42° 8'16.77"N 24°52'17.84"E (2023)
Figure 1. Location of study area and target fields

Experimental design and treatments

Satellite imagery

NDVI was obtained from Copernicus Land Monitoring Service as a daily update of Normalised Difference Vegetation Index provided at pan-European level and in near real time. The data were available at 10 m x 10 m spatial resolution from Sentinel-2 HR multispectral satellite imagery (according to Data viewer - Copernicus Land Monitoring Service).
The images and sample raster values from NDVI were processed by QGIS 3.10.
Time series imageries were downloaded for the same period of experimental in situ data collection and are calculated in days after transplanting the pepper in open field.
Dependencies between the studied indicators were obtained by regression analysis in Excel Microsoft 365 and are valid within the limits of the studied time interval.
In situ data collection was carried out in the period 2022-2023 on a production pepper plantation. The randomized block design was used. Five target fields were determined. These elementary sites are 50 m^2 having 400 plants each (Figure 2).

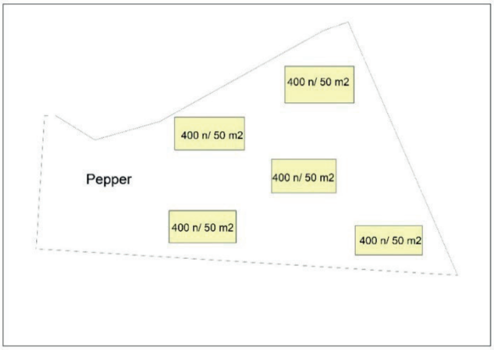


Figure 2. Scheme of target fields

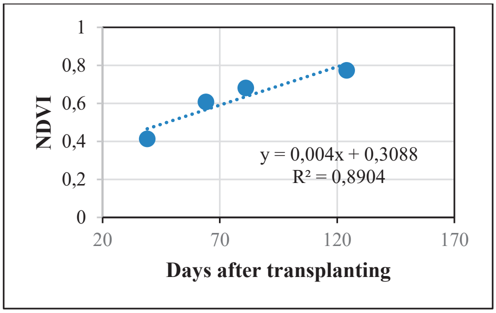
In situ measurements were carried out in four main stages of pepper development - flower buds, flowering, before maturity, maturity. The parameters of growth height of stem (cm) and total fresh biomass (kg/dka) were determined. Height of stem was determined by analyzing 5 plants from each target site. Total fresh biomass was determined as the sum of the weight of leaves and stems for the stages flower buds and flowering and as the sum of the weight of stems, leaves and fruits for the stages before maturity and maturity (Arnaudova et al., 2022).

Pepper agronomy

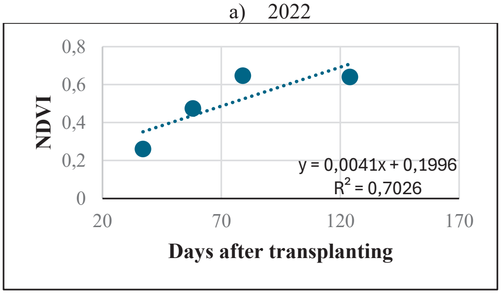
The pepper was grown according to the conventional technology for middle-early field production in open fields (Cholakov, 2009). The scheme used is 90+70/15 with a population of 8333 plants/da and drip irrigation.

RESULTS AND DISCUSSIONS

Among the typical spectral vegetation indices, NDVI is one of the most suitable for tracking crop development dynamics as it measures the photosynthetically active biomass of plants. It can be used throughout the crop production season, except when plant cover is too sparse and therefore its spectral reflectance is too low. NDVI values are most accurate in mid-season, at the stage of active crop growth (https://eos.com/make-an-analysis/). The variation of NDVI was analysed during the growing season of pepper in 2022 and 2023. The relationship between the NDVI values and the condition of the plants in the different stages of their development was established (Figure 3 a, b).



a) 2022



b) 2023

Figure 3. Correlation between NDVI value and pepper development

The determined NDVI values from the satellite images change from 0.41-0.77 for 2022 and 0.26-0.64 for 2023 After the regression analysis, a high degree of multiple correlation R^2 = 0.89 for 2022 and R^2 = 0.70 for 2023 was found.

The results obtained from the *in situ* plant height data were similar for both experimental years. Intense growth of the stem is established in the initial stages of plant development (Figure 4). After the onset of intensive fruiting, vegetative growth slows down.

The dependence of the height of the stems in the different growth stages and the NDVI values for the two years is described by linear equations of the type $y = ax + b$ for both experimental years.

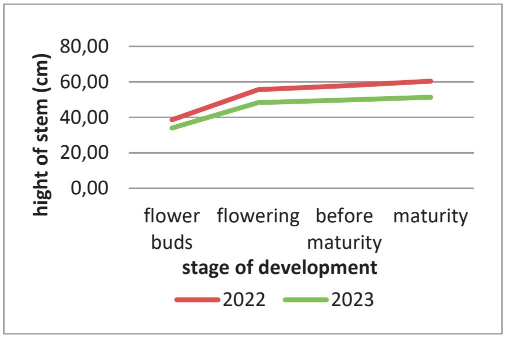
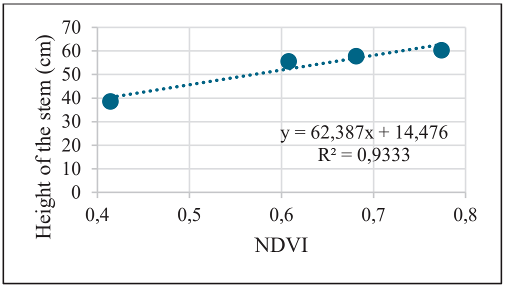


Figure 4. Stem growth dynamics during the different stages of pepper development

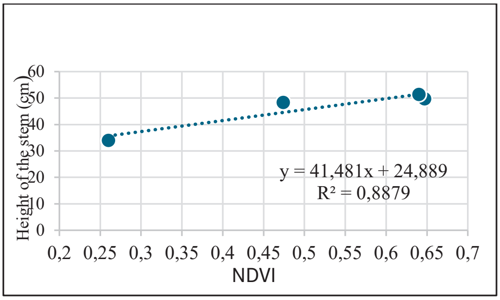
High multiple correlation coefficients R^2 =0.93 for 2022 R^2= 0.89 for 2023 was established (Figure 5 a, b).

The high coefficients of determination suggest that in approximately 90% of the cases, with the established values of NDVI, the height of the plants will be in the indicated trends.

The accumulation of the total fresh biomass follows the trend described in the growth of the stem (Figure 6). More pronounced differences are noted at the beginning of fruit formation. Due to the polycarpic nature of the fruiting of this vegetable species, it maintains constant values in the stages before maturity and maturity. The results obtained by us directly correspond to the specified features of growth and development by a number of researchers (Saban et al., 2014; Panayotov & Jadchak, 2020).



a) 2022



b) 2023

Figure 5. Scatter plot between NDVI value and height of the plant stem (cm)

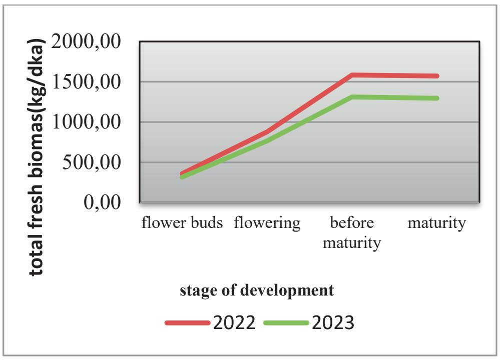
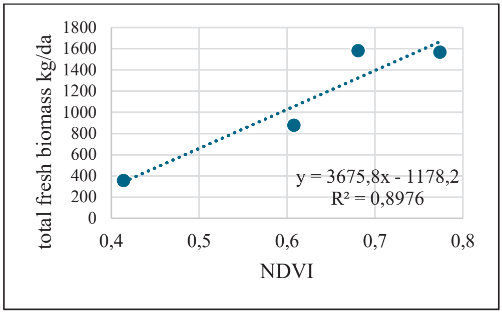


Figure 6. Dynamics of total fresh biomass accumulation during the different stages of pepper development

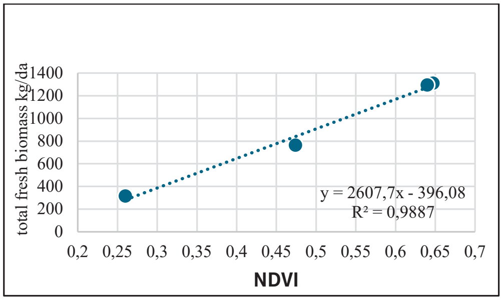
For 2022 and 2023, regression relationships between total fresh biomass, as a complex indicator of productivity, and NDVI were derived (Figure 7 a, b).

The processes are described by a linear regression equation of the *type y = 3675.8 x - 1178.2* and R^2 = 0.90 (2022). For 2023, the equation describing the relationship between in situ data and NDVI is *y = 2607.7x +396.8* and a high degree of multiple correlation R^2 = 0.99.

The high coefficients of determination suggest that in approximately 90% of the cases, with the established values of NDVI the accumulated total biomass will be in the indicated trends.



a) 2022



b) 2023

Figure 7. Relationship between total fresh biomass and NDVI

Shisodia et al. (2020) evaluate the available vegetation indices and find the relevance to plant diagnostics and their use in precision agriculture. They point out that due to the individual nature of their determination (crop-specific vegetation index values) it is necessary to look for a link with analytical measurements that can be performed in situ to validate the data and to prove the relationship between them. The research we have conducted is in the context of their arguments.

CONCLUSIONS

The mathematical description of the established NDVI shows a very good relationship with high degrees of multiple correlations during all development stages of the studied process in pepper.

The established dependencies are in line with increasing stem height and the accumulation of total fresh mass.

The obtained relationship between the *in situ* data and the value of NDVI by time series imagery from Copernicus Land Monitoring Service is the prerequisite for using NDVI as a source of data for pepper growth behaviour.

The guidelines for future research work are: development and testing of regression models based on the developed algorithms for quantification of certain parameters of the studied vegetable crops; development of a comprehensive methodology for monitoring vegetable crops through remote sensing methods.

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INFLUENCE OF IRRIGATION AND FERTIGATION ON SENSORY CHARACTERISTICS OF FRUITS OF WHITE STRAWBERRY (*FRAGARIA X ANANNASSA* "SNOW WHITE") GROWN IN BULGARIA

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Abstract

The aim of this paper is to present the effects of the applied regimes of fertilization and irrigation on the sensory characteristics of white strawberry variety. A two factors experiment was conducted during 2023 in unheated greenhouse in the Chelopechene experimental field, Sofia, Bulgaria with drip irrigated and fertigated strawberry variety (Fragaria x Anannassa "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). Five treatments was tested: control: I0F0:100% (ETc) without fertigation; I1F1; I1F2; I2F1; I2F2. Sensory analyzes were carried out during the first growing season of the fruits according to indicators: appearance, color, consistency, taste, aroma and general sensory evaluation on a 5-point rating scale, given by 10 experts. It was established that the applied agricultural techniques have an impact on the indicators of appearance, color and aroma of the investigated fruit variants. The total sensory evaluation was statistically distinguishable only in the fruits of the control and the variant with 50% irrigation and 75% fertilization (*$p<0.05$*).*

Key words*: strawberry, irrigation, fertigation, sensory characteristics, Bulgaria.*

INTRODUCTION

Strawberries are widely cultivated for their tasty flavor, soft texture, pleasant aroma and nutrient content. In 2022 world production of strawberries was 9.57 million tons produced in 81 countries (FAOSTAT, 2024). At the same time strawberry is one of the most drought sensitive plants due to shallow roots. Water deficit affects yield, vegetative growth and development (Taparauskienė & Miseckaitė, 2014). Soil types and fertilization also have an influence on strawberry quality and sensory characteristics (Taghavi et al., 2019). *Fragaria x Anannassa* berries are an important source of vitamins, minerals and fibre, as are other fruits and vegetables. The diversity of bioactive compounds, such as phenols in berry species, is reflected in a wide range of their biological activities, which have a beneficial effect on human health and disease prevention (Moyer et al., 2002). 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 grow in two botanical forms (Grez et al., 2020) - wild (*Fragaria chiloensis* ssp. *chiloensis* f. *patagonica*) and cultivated (*Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis*). It was brought to Europe in the 18th century. "Snow White" variety has been selected in 2010 out from *Fragaria ×ananassa* "Weisse Ananas" and *Fragaria chiloensis* f. *chiloensis* (Olbricht et al., 2013).

Investigations of sensory characteristics (appearance, color, texture, flavour, odour) of strawberries are conducted mainly for red varieties (Kampuss et al., 2021; Darbellay et al., 2002; Testoni & Nuzzi, 2006; Carlen et al. 2007; Bianchi et al., 2017). White varieties are studied mainly in regard to physiological process (Grez et al., 2021), floral differentiation (Grez et al., 2017), taste and health relating compounds (Sarıdaş et al., 2021), pomological characteristics (Antonova & Petrova-Branicheva, 2023).

The aim of this paper is to present the effects of the applied regimes of fertilization and irrigation on the sensory characteristics of white strawberry variety *Fragaria x Anannassa* “Snow White” based on the harvest date.

MATERIALS AND METHODS

A first year two-factor experiment was conducted on drip irrigated strawberry plants in an unheated polyethylene tunnel greenhouse in 2023 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, agro technologies and plant protection “Nikola Pushkarov” in Sofia, Bulgaria (Figure 1). Mulching was applied to further reduce water evaporation and inhibits weeds. The object of the study was white strawberry variety (*Fragaria x Anannassa* “Snow White”).

Figure 1. View from unheated polyethylene tunnel greenhouse in the Chelopechene, Bulgaria

The experimental treatments were arranged according to the method of long plots. Each treatment was replicated three times. 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). Five treatments were tested: control treatment I0F0: 100% (ETc) - full irrigation and without fertigation; I1F1; I1F2; I2F1; I2F2. The microclimate of greenhouse: 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 and recorded in data logger (HOBO USB Micro Data Logger, USA). FAO Penman-Monteith Equation (Allen et al., 2006) was used for determining reference evapotranspiration and irrigation scheduling. 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 fruits during the first vegetation period in three harvests (6 June, 22 June and 4 July) have been provided and analysed in the Food Testing Laboratory at the Institute of Food Preservation and Quality in Plovdiv, using an adapted methodology developed at the same institute.

On the day of harvesting, the fruits were transported in coolers, and the evaluation was conducted 24 hours after the harvest was brought to the Sensory Laboratory at a temperature of 20 ± 5°C. Ten trained experts were provided with coded samples of strawberry fruits from the four cultivation variants and the control for assessment. During the sensory analysis, the tasters neutralized their taste buds between sample variants with water and crackers.

To assess the sensory attributes of the fruits for the three harvests, the tasters provided their quantitative ratings on a five-point hedonistic scale for the following indicators: external appearance (uniformity and size of the fruits), color (white with red seeds), consistency (juiciness, presence of seeds), taste (sweetness, taste intensity), and aroma (fruitiness, aroma intensity). The overall sensory evaluation is calculated based on the sum of the average ratings given by the tasters, multiplied by the weighting coefficient for each category: external appearance - 0.25; color - 0.20; consistency - 0.15; taste - 0.25; and aroma - 0.15.

Mathematical and statistical analysis

The presented results are the arithmetic mean values from at least three parallel determinations, with variation coefficients less than 5%. The statistical data processing was conducted using ANOVA software, as well as Microsoft Excel and SPSS Statistics.

RESULTS AND DISCUSSIONS

Three series of tests were conducted on the investigated variants of white strawberries depending on the harvests. The data is presented in Figure 2. The fruits from the first harvest of the control group (I0F0) are uniform, with average dimensions 29.6/25.2 mm and average weight 8.55 g (Figure 3), white in color with red seeds, juicy, sweet, and aromatic. The white strawberries from the second harvest had average dimensions 21.9/19.5 mm and average weight 4.42 g, white in color with pink hues in some areas, with red seeds. They are juicy but lack sufficient sweetness and have a weak aroma.

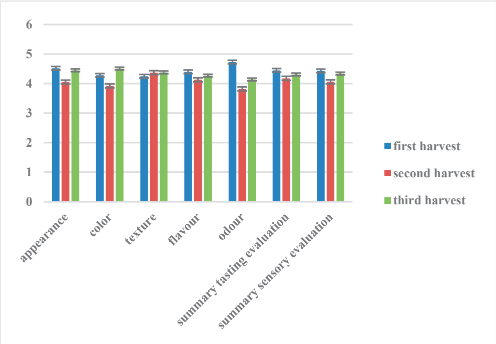

Figure 2. Sensory characteristics of white strawberry fruits in the control group (I0F0) depending on the harvests

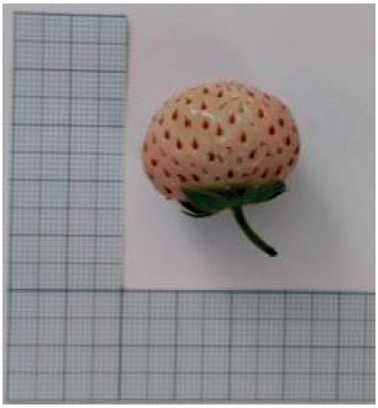

Figure 3. White strawberry fruits from the control group (I0F0)

During the third harvest, the fruits had average dimensions 21.9/19.2 mm and average weight 4.52 g, white in color with pink hues and red seeds. They are juicy, with sweetness and aroma intensity close to those of the fruits from the first harvest. For the control variant of white strawberries, the external appearance of the fruits received very good ratings for the first and third harvests, while for the second harvest, the tasters provided good ratings. Similar results were obtained for the evaluations of color, overall taste, and overall sensory assessment. The factor of harvest time influences the values of these indicators ($p < 0.05$).

For the consistency indicator of the investigated fruits, it was found that the harvest factor does not have an influence, and the ratings (above 4.2) are statistically indistinguishable ($p > 0.05$). The tasters rated the taste of the fruits with good scores, with fruits from the first harvest leading, followed by fruits from the third harvest. Fruits from the second harvest received the lowest scores for taste (4.13), indicating that the harvest time influences the taste of the fruits ($p < 0.05$).

With maximum and minimum ratings for all examined fruits and indicators, the evaluators gave the aroma indicator for white strawberry fruits as follows: for the first harvest (4.73) and for the second harvest (3.82). After the statistical processing of the data, it was confirmed that the harvest time factor influences the aroma of the fruits ($p < 0.05$). The overall taste assessment and the formed overall sensory evaluation of the white strawberry fruits from the control group showed that there were no statistically significant differences between the first and third harvests (values above 4.3) ($p > 0.05$), while the harvest factor influences the ratings of the fruits from the second harvest ($p < 0.05$).

The data from the conducted sensory characteristics of the white strawberry fruits, variant I2F1, are presented in Figure 4. The fruits from the first harvest had average dimensions 29.2/24.7 mm and average weight 8.23 g (Figure 5), white with red seeds. Fruits from the second harvest had average dimensions 21.9/18.4 mm and average weight 4.04 g and third harvest had average dimensions 2.02/18.6 mm and average weight 3.68 g, a pink hue, better consistency, and juiciness.

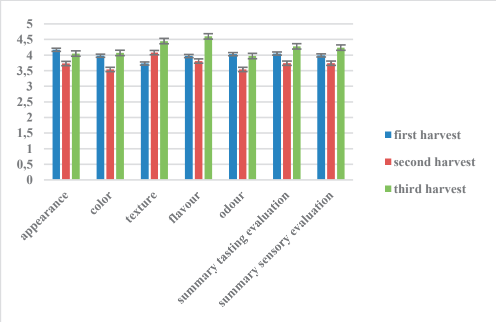

Figure 4. Sensory characteristics of white strawberry fruits, variant I2F1, depending on the harvests

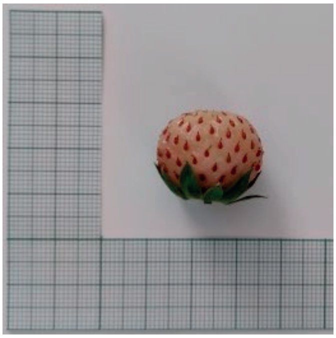

Figure 5. White strawberry fruits from variant I2F1

For all examined fruits, the ratings for the indicators of external appearance, color, and aroma are statistically indistinguishable between the first and third harvests. The harvest time factor does not influence these indicators of the fruits, and they are around and slightly above 4 ($p > 0.05$). With maximum ratings (4.5), the tasters evaluated the fruits from the third harvest for consistency and taste indicators, while with minimum ratings (3.5) they evaluated the fruits from the second harvest for indicators such as external appearance, color, aroma, taste, overall taste assessment, and overall sensory evaluation. The harvest time factor influences the examined indicators of the fruits from the second harvest ($p < 0.05$), while the fruits from the third harvest have maximum or similar values to those from the first harvest. White strawberry fruits grown with 75% irrigation and 100% fertilization (I1F1) from the first harvest had average dimensions 30.4/26.2 mm and average weight 8.99 g (Figure 6), white with red seeds, juicy, moderately sweet, and aromatic. From the second harvest, the fruits are smaller, average dimensions 22.5/20.2 mm and average weight 4.54 g, white with pink hues, red seeds, juicy, with insufficient sweetness and aroma. The white strawberries from the third harvest had average dimensions 23.7/20.1 mm and average weight 5.22 g, uniform, white with pink hues, red seeds, juicy, sweet, and aromatic.

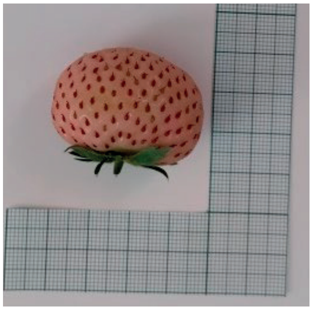

Figure 6. White strawberry fruits from variant I1F1.

The fruits from the first and third harvests have the highest average ratings for the examined indicators and the summarized taste and sensory evaluations (Figure 7). Once again, the fruits from the second harvest have the lowest average ratings, and the harvest time factor influences their sensory indicators ($p < 0.05$).

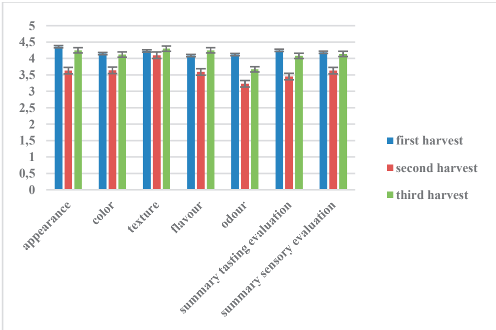

Figure 7. Sensory characteristics of white strawberry fruits, variant I1F1, depending on the harvests

Figure 8 shows the average ratings for the examined sensory indicators of fruits grown with 75% irrigation and 75% fertilization (I1F2). The fruits from the first harvest had average dimensions 30.4/26.2 mm and average weight 8.59 g (Figure 9), white with red seeds. Fruits from the second harvest had average dimensions 22.5/19.8mm and average weight 4.47 g, white with a pink hue, red seeds, juicy, but with insufficient sweetness and aroma. Third harvest had average dimensions 23.5/19.4 mm and average weight 5.02 g, a pink hue, better consistency, and juiciness.

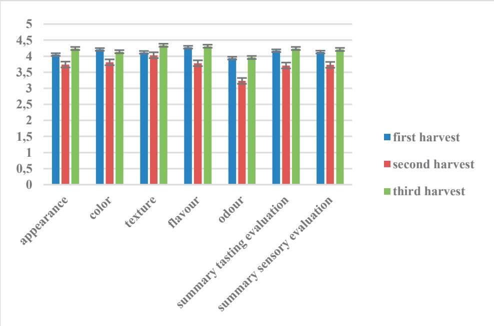

Figure 8. Sensory characteristics of white strawberry fruits, variant I1F2, depending on the harvests

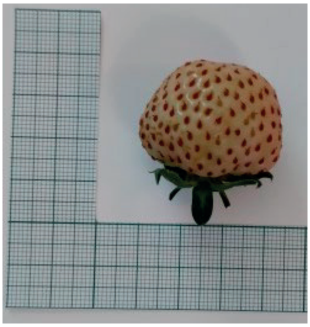

Figure 9. White strawberry fruits from variant I1F2

The tasters gave good ratings for the fruits from the first and third harvests, while they rated the fruits from the second harvest with slightly lower scores. Comparative analysis showed that for all indicators of the fruits from all harvests, the aroma was rated lower than (3.2). White strawberries from the first harvest, grown with 50% irrigation and 75% fertilization (I2F2), are small-sized, average dimensions 27.9/24.1 mm and average weight 7.16 g, white with red seeds, softer in texture, sweet, and aromatic. The fruits from the second harvests are white with a pink hue, red seeds, juicy, sweet, and slightly aromatic had average dimensions 21.6/18.4 mm and average weight 4.04 g (Figure 10). The fruits from the third harvest had average dimensions 19.5/17.2 mm and average weight 3.39 g.

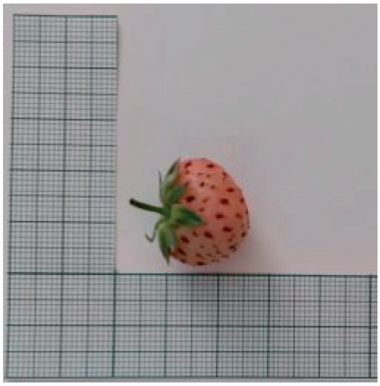

Figure 10. White strawberry fruits from variant I2F2

The fruits grown under this variant were rated with average scores across the three harvests for the indicators of external appearance, color, aroma, overall sensory assessment, and taste assessment (Figure 11).

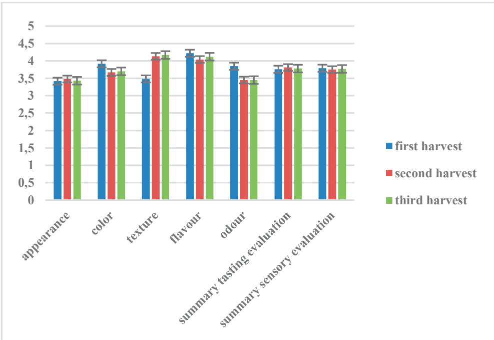

Figure 11. Sensory characteristics of white strawberry fruits, variant I2F2, depending on the harvests

Only for the indicators of taste and consistency did the tasters rate the fruits with good scores for all three harvests. The strawberry fruits from the first harvest received good ratings for color, taste, and aroma, with a minimum rating for consistency. The fruits from the remaining harvests had statistically indistinguishable values among themselves, and the harvest factor did not influence the mentioned indicators. After statistical data processing, it was found that for the indicators of external appearance, overall taste assessment, and overall sensory evaluation, the fruits from the three harvests showed no statistically significant differences, and the harvest factor did not influence these indicators ($p > 0.05$).

The comparative analysis for all variants, depending on the applied irrigation and fertilization treatments of white strawberry fruits, showed that after applying statistical methods such as Principal Component Analysis (PCA), ANOVA, and Pearson Correlation, significant differences were found in the examined sensory characteristics and overall sensory evaluation. The fruits from the control group I0F0, and the fruits from I2F2 showed these differences. Negative linear correlation dependencies were identified for all cultivation variants of white strawberries, with a moderate coefficient of determination (R^2 = 0.549), between sensory color evaluations and color saturation calculated from instrumental measurements of the fruits, as well as between sensory color evaluation and calculated color index (R^2 = 0.624). The influence of the cultivation method on the taste for all examined fruit variants was established through a negative linear correlation with a high coefficient of determination (R^2 = 0.806) between soluble solids content and sensory taste evaluations.

CONCLUSIONS

A sensory evaluation was conducted on white strawberry fruits grown under different irrigation and fertilization regimes (I1 - 75% (ETc), I2 - 50% (ETc), F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 - suboptimal fertilization - 75% (F1), with five treatments tested: control: I0F0: 100% (ETc) without fertilization; I1F1; I1F2; I2F1; I2F2) at three harvest maturities (6 June, 22 June and 4 July). It was found that the applied agronomic techniques influence the indicators for external appearance, color, and aroma of the studied cultivation variants' fruits. The overall sensory evaluation is statistically different only for the fruits from the control group I0F0 and the variant I2F2 ($p<0.05$). The harvest factor influences the sensory characteristics of the fruits for all cultivation variants. The fruits with maximum sensory scores for the examined indicators are those from the first harvest of the control group (I0F0), I1F1, I1F2, and I2F2, followed by the white strawberries from the third harvest. The fruits from the second harvest have the lowest taste ratings depending on the harvests for all examined agronomic technique variants.

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CLIMATE CHANGES AND ITS EFFECTS ON SOILS AND AGRICULTURE IN WESTERN AND SOUTH-WESTERN ROMANIA

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Abstract

The paper refers to certain aspects of risk that have influenced the climate changes of the last two decades, especially after the year 2000. Updated data from the representative meteorological stations belonging to the Banat-Crişana Regional Meteorological Centre are presented. More extensive research was carried out in the case of thermal regime, pluviometric regime, and wind regime, regarding the evolution and deviations of these parameters in western and southwestern Romania compared to the national level. From 1960 until now, average temperatures have increased by up to 1.5°C in the Banat area and by 2-3°C in southern Romania, especially in the summer months. It is also worth noting the anomalies that occurred after 2000. At national level, 2019 was the warmest year. In the period 2012-2022, the most positive thermal anomalies were recorded (0.7-1.9°C), making it the warmest period of 10 consecutive years in the history of meteorological measurements recorded in Romania, with negative effects on soil cover and agriculture.

Key words: *climate change, thermal regime, pluviometric regime, soil cover, agriculture.*

INTRODUCTION

At the beginning of soil research, the theories supporting these phenomena were based on one or more pedogenesis factors, almost always different, a fact that favoured the emergence of a multitude of hypotheses or concepts Drăghici, 1988.

Among the first scholars who did research on soils were the ancient Greeks and Romans: Aristotle, Cato, Collumela, Hippocrates, Plinius, Varro, and Virgilius (Ianoş, 2004). Notions related to soil, still valid today, can also be found in treatises from the Middle Ages: Agricola (1494–1555), Lister (1683), Leonardo da Vinci (1452–1529), Paracelsus (1493–1541), while the first scientific work of scope was Agriculturae Fundamenta Chemica by the Swede Wallerius from Upsala (1761) (Bogdan & Niculescu, 1999).

Modern approaches to soil genesis processes, which integrate the soil into the set of environmental factors, arguing and quantifying the contribution of each external intervention, appeared much later, towards the middle of the 20th century (Brady & Weil, 2003; Cresser et al., 1993).

The explanation of soil formation cannot be limited to the interaction between the biosphere and the lithosphere, excluding geological processes. Thus, Erhart (1967), through his theory known as Biorhexistasia, stated that, "in geological times, successive periods of formation and destruction of the soil cover were distinguished. During periods of tectonic calm (biostasis), the soil cover was formed and evolved." During periods of imbalance (erosions, tectonic movements, volcanism), previously formed soils are destroyed (Sărățeanu et al., 2023). According to this theory, the soil cover evolved cyclically, with periods of interruption and resumption of the process, which explains its heterogeneity and diversity. This concept is supported by Gerrard (1981) who, quoting Butler, states that buried soils represent exactly the evolutionary cycles between the different phases of tectonic or climate instability (Alda et al., 2011; Barbu & Popa, 2003).

In Romania, in the study of soil formation and evolution phenomena, three distinct periods were identified: before 1906, between 1906 and 1948, and after 1948. Before 1906, pedological research was not organized and no well-defined concept was stated (Mihuț et al., 2022). It started with the chemical, physical, agro-geological conceptions, taken from Western Europe literature, then, after the International Congress of Geology in Petersburg in 1897, Matei Drăghiceanu brought to the country the

views of the Russian naturalist school which, gradually, took the place of the German agrogeological school views.
The initiator and organizer of the Romanian geographic-naturalistic soil genesis school was the geologist Gheorghe Munteanu Murgoci, who recommended studying the soil in close connection with the factors and processes of soilification. He stated that, to understand the formation and evolution of soils, the action of any factor should not be taken separately, independently of the action of the other factors (Mihut et al., 2022). In his view, the most important factors in the formation and transformation of soils were: parent rock, water, climate conditions, vegetation, and animals (Dobrei et al., 2016).
Climate influences both directly and indirectly the formation and evolution of soils through a complex of factors such as: temperature, precipitation, global radiation, cloudiness, relative humidity, evapotranspiration, etc. (Sircu, 1971; Tarca, 1998). Temperature influences the intensity of all soil processes: adsorption, alteration, mineralization, humification, evapotranspiration (Mahara, 1979; Mihailovici et al., 2000; Mircov et al., 2023; The climate of Romania, 2008).
The soils that form and develop in constant and uniform climate conditions are monogenetic – such as the soils of Banat formed after the last glaciation (Nichita, 2011).
The studies of Romanian researchers have contributed to the refinement of regional projection methodologies of the global warming signal (Povară, 2006). Statistical modelling methods applied to the results of global climate models were used, but also numerical experiments with regional climate models and the analysis of their results together with the observed data to highlight mechanisms by which local factors modulate the global signal of climate change (Pop, 1988; Posea, 2006).
Very short-term forecasting was done for small intervals of 12 hours up to 2-3 hours from where the nowcasting starts. The information underlying these weather forecasts was provided by the national radar system and the lightning detection system, images provided by meteorological satellites and information from automatic surface stations (Mircov et al., 2022).
Meteorological phenomena were permanently monitored by each Regional Weather Forecast Service and the Nowcasting Collective, elaborating forecasts for intervals of less than 12 hours, but also warnings regarding large amounts of precipitation, storms, or hail.
There was also the nowcasting which represents the provision of information about the occurrence of a meteorological phenomenon such as lightning, hail, heavy rains, wind intensifications. The amount of information provided by the nowcasting was conditioned by the ability to anticipate and detect the respective phenomenon. Lightning and tornadoes are phenomena that can be anticipated only minutes before they occur; however, their occurrence can be detected in real time with the help of lightning detectors and radars.
We constantly analyse climate fluctuations and we develop projections of the evolution of the climate system both on a large scale and on the scale of Romania, transferring scientific knowledge to the socio-economic environment through climate products and services.
From an agrometeorological point of view, February 1 is a reference moment for the start of thermal summations, since the analysis of the thermal oscillations of the last decades shows that the sporadic warmings (windows) during the month of February influenced the resumption of the vegetation cycle. Berbecel (1979) claims that, in 80% of cases, the resumption of the vegetative cycle in wheat begins in February.
For most cultures, Berbecel claims that the thermal index of de-springing is between 100°C and 500°C and it is achieved in the period February 1 II - April 10. Against the background of cold air invasions from the north-eastern areas of Europe, in the western part of Romania, especially in the hills, the last days with frost were reported in the second decade of April and very rarely later (Archive CMR Banat-Crișana).
Most authors, including Marchiș (2018), state that, "the temperatures characteristic of the spring season were particularly important because atmospheric conditions have a decisive influence on the state of vegetation of winter and spring crops, a fact that influences the beginning of the agricultural campaign".

In the southwestern part of Romania, the wind regime is determined by the development of baric systems that interfere over Europe at the latitude of 4500 N (Azoric, Siberian, Scandinavian anticyclones, and Mediterranean, Icelandic cyclones) (Moldovan, 2003; Munteanu, 2001; Nichita & Hauer, 2010).

Concerns regarding the soil mapping of some areas in Banat appeared relatively recently, after 1960. Based on the numerous information collected over the years, in 1993, Ianoş, Puşcă & Ţărău recreated the soil map of Timiş county on a scale 1:50,000. Ianoş (1994) first elaborated the map, after which Rogobete & Ţărău (1997), as well as Ianoş & Pușcă (1998) published the "Banat Soils Map" at a scale of 1:100,000, a synthesis of the work of several generations of pedologists grouped under the Office of Pedological and Agrochemical Studies from Timișoara (Mihuț & Niță, 2018).

MATERIALS AND METHODS

The purpose of this paper is to report certain meteorological interpretations on the thermal regime, the pluviometric regime and the wind regime characteristic of the western part of the country in the last twenty years (Drăghici, 1988; Ghibedea & Băcanu 1982; Mircov et al., 2017). Also, certain interpretations were made on synoptic maps. To best describe this aspect, we took into account the meteorological data provided by the Banat Crișana Regional Meteorological Centre, more precisely, the data from representative meteorological stations in Timiş, Arad, and Caraș-Severin counties. The more detailed analysis of certain phenomena was done to capture exceptions, special situations, or more significant meteorological elements (Mircov et al., 2023). For the climate characterization of the studied area, the records made at the level of the studied locality were also used (Archive CMR Banat-Crișana).

To achieve a correlation between the meteorological interpretations specific to the Western area of Romania and the influence these data have on the formation and evolution of soils and agriculture in general and for the best possible interpretation between the various studies carried out over the 20 years, it is necessary to detail the working methods and methodologies so that, in the future, comparisons can be made between the present results and those carried out according to other methods.

Regarding the soil study, a series of field trips were made, observations were made, soil samples were collected and soil properties were analysed, after which the data were processed according to the methodology in force. Given that the soil is a complex system that requires an increased diversity of methods and procedures for study, some general, others adapted from related sciences and others specific to the field. All study methods have a common starting point, namely - the elementary territorial entity of soil, which was investigated by soil researchers and especially by OSPA.

The analyses and other determinations were carried out in the laboratories of the Offices for Pedological and Agrochemical Studies in Timișoara and Arad.

RESULTS AND DISCUSSIONS

The soil cover in the western part of Romania presents the same arrangement in steps, from east to west, as the relief, the climate, or the vegetation. This characteristic is associated with the horizontal zonation of the soil cover, starting from the plain area, which gradually turns into a vertical zonation in the hill and mountain regions. This stepwise arrangement of the soils is primarily related to the formation of the relief in the western part of the country, through the appearance of dry land under the waters of the sea, from east to west. Because of this, the zonation of the soils follows the line of the meridians and is, generally, the same towards the piedmont and mountain area.

Table 1 presents the main soil types and associations of the studied area.

Table 1. Main types and associations of soils in Western Romania

No	SRTS - 2012
1.	Lithosol and folisol (di, eu, pr, rz)
2.	Regosol (di, eu, mo, um, li)
3.	Psamosol (eu, mo, gc)
4.	Aluviosol (en, eu, mo, gc, vs, sc, ac)
Protisols	
5.	Chernozem (ti, gc, ka,vs, sc, ac)
6.	Phaeosiom (ti, vs, gc, st, cl)
7.	Rendzine (li, cb, ka)
Chernisols	
8.	Eutricambosol (ti, mo, vs, ro, al)
9.	Districambosol (ti, um, ep, li)
Cambisols	
10.	Preluvosol (ti, mo, rs, vs, ca, st)
11.	Luvosol (ti, rs, ab, vs, pe, st)
12.	Planosol (ti, ab, vs, st)
Luvisols	
13.	Vertosol (ti, gc, st, br)
14.	Pelosol
Vertisols	
15.	Gleysol (eu, di, ka, mo, ce, ca, pe, al)
16.	Stagnosol (ti, lv, ab, vs, pl)
Hydrisols	
17.	Soloneţ (ti, mo, lv, ab, sc, gc)
Salsodisols	
18.	Erodosol (ca, cb, ar, sp, li)
Antrisols	

The southwest of Romania, like the whole country, due to its geographical position – in the temperate zone – is exposed to a wide range of meteorological and climate risk phenomena with the potential to occur throughout the year.
The purpose of this work is to identify some indicators that most correctly express the extreme nature of the manifestations of some meteorological parameters and their characterization in the 20-year interval, the 2018-2021 interval, it was noted by more accentuated anomalies within the pluviometric regime, and in the years 2017 and 2021, the wind regime was most evident in the south-western area of Banat, a region where the Coșava wind had an intensity of up to 100-110 km/h.
The main meteorological parameters from the representative stations in the south-west of the country were analysed to have an overview of the weather-climate characteristics. The more detailed analysis of certain phenomena was done to capture exceptions, special situations, or more significant meteorological elements.
The location of Romania in a certain context of action of the main baric centres imprints the temperate character of the climate. The location of the analysed territory in the south-western part of the country and the configuration of the relief define this character, imprinting sub-Mediterranean characteristics in the southern extremity and oceanic in the northern and eastern part of the area.
Figure 1 shows the map representing the entire hydrological network of the area.

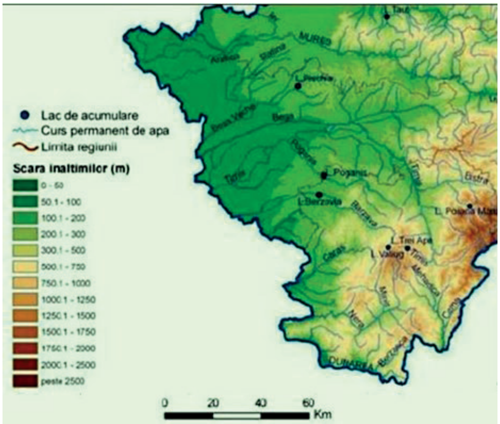

Figure 1. Map of the hydrographic network (from www.adrvest.ro)
(Legend: Permanent water course; Region limit; Height scale - m)

Knowing the weather-climate characteristics of the region is important for all areas of human activity and life, all extreme phenomena grafting on the main background of the mentioned weather-climate characteristics (Archive CMR Banat-Crișana).
Most of the climate indicators from the analysed period were presented for the Western part of Romania, considering the thermal regime, the pluviometric regime, and the atmospheric pressure.
The hottest month of the year was July, with average temperatures of 21^{0}C, and the coldest was January, when the average temperature reached 2^{0}C (Figure 2).
The annual average temperature variation has approximately the same value at all stations, the amplitude and values encountered differ. The highest values in the summer months were found at the southern stations, where the average temperature was over 22^{0}C at Moldova Veche and Banloc. The lowest average temperatures were recorded in the Bozovici depression area, where the temperature did not reach 20^{0}C (except for the stations in the mountain area).

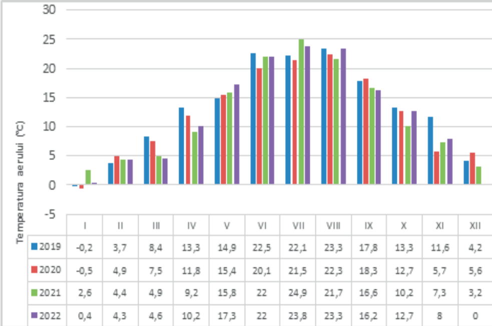

Figure 2. The evolution of the thermal regime in the period 2019-2022 from the town of Lugoj, Timiș county

From a thermal point of view, the climate specific to the western part of the country is also felt in this area, the hottest of the last studied years was also in Lugoj, a locality located 50 km from Timisoara, especially in the years 2019 and 2020. in November of 2019 we had frequent days with temperatures that exceeded 20^{0}C, and the monthly average for this month was 11.6^{0}C. Only in January was the monthly average negative, -0.2^{0}C, in January of 2019, respectively -0.5^{0}C, value recorded in the following year, in 2022. The summer of 2021 was the warmest, the highest monthly value was recorded in July, more precisely, 24.9^{0}C.

The month of January was the coldest month, with averages between 0 and -3^{0}C, the lowest value was recorded in the Bozovici Depression and in the Mureșului Corridor at the Vărădia de Mureș station, against an inversion background, and the highest values were recorded in the south, in Oravița and Moldova Veche. The mountain area recorded the lowest temperatures, a normal fact due to the altitude, the average in January reaching -8^{0}C in Țarcu.

For the analysed interval, in the analysed area, except for the mountainous area, records average values that exceeded 22^{0}C in the months of July, the highest value being registered in 2021, respectively 25.7^{0}C (Fig. 3).

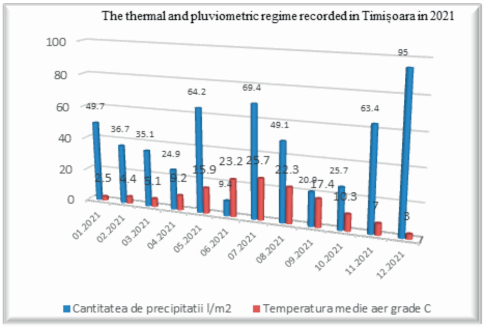

Figure 3. The thermal and pluviometric regime recorded in Timișoara in 2021
(Legend: amount of precipitation - l/m^2;
average air temperature degrees - ^{0}C)

In the studied region, spring was somewhat earlier and warmer compared to other areas of the country. Temperature oscillations occur with colder periods under the influence of air masses from the north and northeast, but also warmer periods due to the activity of Mediterranean cyclones. Thus, late frosts and isolated frost can occur on the coldest mornings even at the beginning of May, but hot days also occur in June. Also, in the spring, the first convective manifestations appear with stormy phenomena, torrential rains, and hail. Average temperatures gradually increase from 5-6^{0}C at the beginning of spring to 16-17^{0}C at the beginning of summer. Annual average values range from 7 to 11^{0}C.

Summer was dominated by formations related to the Azorean anticyclone and the Mediterranean cyclones, starting early, sometimes even in May and lasting until September. The average temperature of the hottest month was July and it varies between 21-22^{0}C in the Oravița-Moldova Veche area and 8^{0}C at high altitudes. Hot days occur with highs exceeding 35^{0}C and tropical nights with lows over 20^{0}C. For the analysed interval, it can be observed that, in Timişoara, in the month of July, the average values exceeded 22^{0}C, the highest value being registered in 2021, which was 25.7^{0}C. This year, 16 hot days were recorded in Timişoara in the summer months. Compared to 2021, in 2018 there were 11 hot days during the summer (Figure 4).

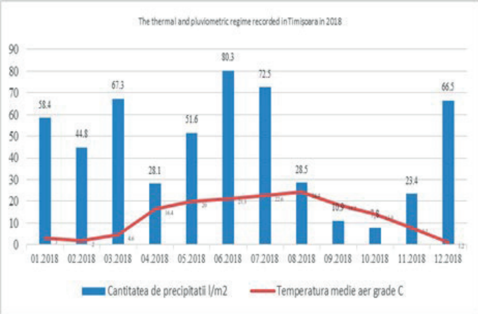

Figure 4. The thermal and pluviometric regime recorded in Timișoara in 2018
(Legend: amount of precipitation - l/m^2; average air temperature degrees - ^{0}C)

From the point of view of the pluviometric regime, it can be observed that the least precipitation in the analysed period was recorded in the months of October, November, and December of 2019, during which the precipitation reached only 57 l/m^2. This period also continued in the spring of 2020, a 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 maize crop production (Figure 5).

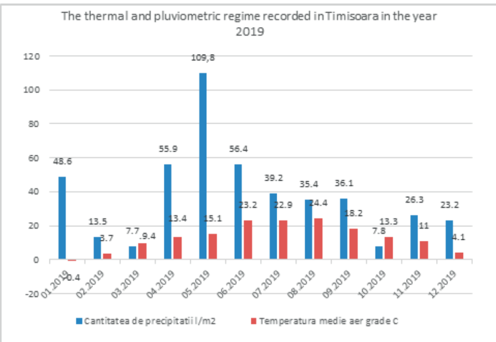

Figure 5. Thermal and pluviometric regime recorded in Timișoara in 2019
(Legend: amount of precipitation - l/m^2; average air temperature degrees - ^{0}C)

Starting from June 2020, when values of 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 (Figure 6).

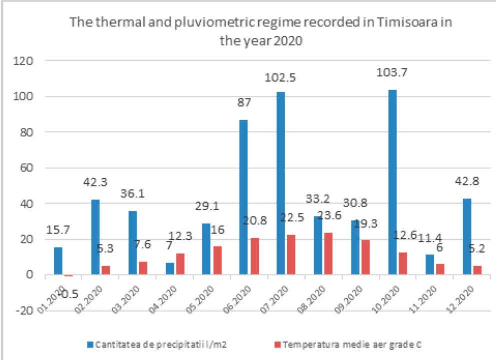

Fig. 6. Thermal and pluviometric regime recorded in Timișoara in 2020
(Legend: amount of precipitation - l/m^2; average air temperature degrees - ^{0}C)

For the analysed interval, a decrease in the rainfall regime in Timişoara was observed, the average slightly exceeded 510 l/m^2, the driest year being 2019, a year in which it rained a little over 460 l/m^2. The exception was May with 109 l/m^2, after which a dry period followed until November, a month in which 26 l/m^2 were recorded.

The annual values of the atmospheric pressure in Timişoara give a multiannual average of 984.4 mb, the atmospheric pressure at the station level, i.e. the pressure read at the barometer, to which temperature and gravity corrections have been applied. In 2018 and 2020, an average below the value of 980 mb was recorded, which means a more intense cyclonic activity and, implicitly, a higher number of cases for the respective years with manifestations of the wind (Figure 7).

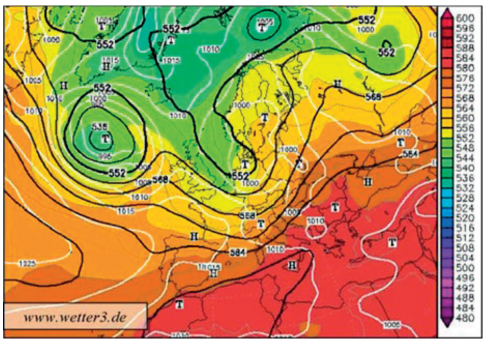

Figure 7. Ground atmospheric pressure and geopotential at 500 hPa

The shining duration was an indicator that represents the time interval during a day in which the sun shone on the sky. This consists of determining the number of hours during which the sun lighted the meteorological platform and its surroundings and depends on cloudiness, latitude, season, and altitude. The annual average was around 2,000-2,100 hours, an interval also found in the analysed period. The maximum annual value reached over 2,500 hours in 2000 in Timişoara, and the minimum 1,580 hours in Reșita in 1980. The highest average value was recorded in July or August and the minimum in December, less often in January. The most hours of sunlight were recorded in July 1963, in Timişoara, 290 hours to be exact.

The factors that determine the frequency and speed of the wind are related to the general circulation of the atmosphere, to which are added the influences of the local circulation. The magnitude of baric and thermal gradients related to baric formations, convection, all influence wind speed. Westerly winds predominate, with particularities determined by the above factors. Atmospheric calm prevails, in percentages varying between 21.5% in Sânnicolau Mare and 66% in Vărădia de Mureș.

In the images below (Figures 8 and 9), the wind rose for two representative stations from Caraș-Severin county, the one from Herculane and the one from Bozovici, respectively from Timiş county, the Sânnicolau Mare station, and the one from Lugoj are presented; Figure 10 shows graphically the relative humidity (%) of some more representative years from the studied period, namely the years 2002, 2007 and 2014.

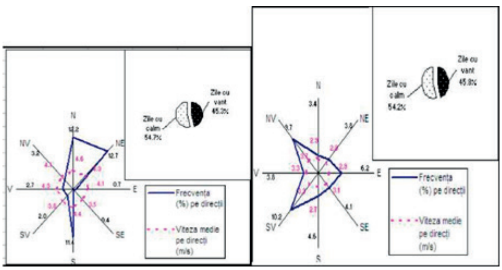

Figure 8. Win rose in Herculane and Bozovici (after, Nichita, 2011)

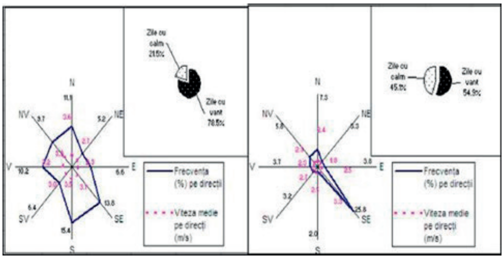

Figure 9. Win rose in Sânnicolau Mare and Lugoj (after Nichita, 2011)

From the images presented, the predominant wind direction was SE at Lugoj, while at Herculane the prevailing direction was N and NE.

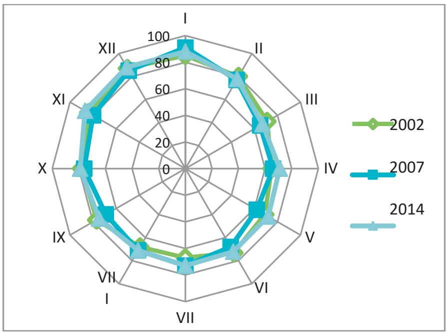

Figure 10. Graphical representation of relative humidity (%) in the years 2002, 2007 and 2014 at Timişoara Meteorological Station

Figure 10 shows how the relative humidity recorded at the Timişoara Meteorological Station had values between 20-60%. It kept on like that: higher values were found in the winter than in the summer, when the values decreased by a few percents.

CONCLUSIONS

The analysis of the climate conditions in the western part of Romania highlights the following aspects:

- The Banat area constitutes a favourable environment for the development of most agricultural crops specific to this latitude and altitude;
- Large temperature fluctuations between day and night, during winter and at the beginning of spring, cause great damage to fruit trees;
- In dry years, with high temperatures, plants suffer from temporary withering;

- In the spring, late frosts were harmful, which surprise the still undeveloped plants and which also affect the orchards;
- The amount of precipitation, even if it is sufficient, in most years has an uneven distribution (there were periods of drought even in the critical phases of vegetation development), affecting the growth of crops;
- Torrential rains were more frequent in the months of May and June, when they cause much more damage, compared to the rest of the year;
- Except for the area around Timișoara, the winds have low frequencies and intensities, which does not require the planting of protective curtains;
- The relative humidity values allow the successful cultivation of vegetables, fruit trees, vines, and crops;
- Soil fertility and their productive capacity intervene in the genesis process and influence the favourable environmental conditions for the growth and development of plants;
- The heat from the soil surface leaves its mark on the intensity of evaporation from the soil surface and is necessary to consider when measuring the rate of evapotranspiration;
- The precipitation, close to the average value at the level of the country, supplies the soil with water and it acts, depending on the local conditions, on specific soil genesis processes such as: eluviation, illuviation, pseudo-gleysation, in different ways (percolation, stagnation, silting, draining);
- The combined action of temperature and precipitation influenced the formation of clay minerals in soils, so that, over time, the proportion of resulting clay minerals increased proportionally with humidity and exponentially with temperature;
- The gaseous phase occupies a percentage of 5-40% in the soil, lower in the compacted and finely textured soils and maximum in the loose and coarsely textured soils of the area;
- The soil reflects the climate condition through the way and intensity of alteration of the mineral part, through the leaching intensity of some mineral constituents or through the nature and intensity of the decomposition of organic matter.

Thus, there was great non-periodic variability of dryness and drought in frequency, duration, and intensity. According to the opinion expressed by Bogdan (1980), 22 periods of drought occurred in the Western Plain. In the relatively small number of drought periods compared to other regions of the country, an important role was played by the geographical location of the Banat area in relation to the main air masses that affect Romania.

As mentioned in the paper, with regard to the precipitation values during the analysed period, recent examples of drought were reported in the spring of 2007, when several weather stations accumulated amounts of less than 1 l/m^2 in April or at the end of the autumn of the year 2011.

The annual average was around 2,000-2,100 hours, the annual maximum can reach over 2,500 hours as in 2000 in Timișoara, and the minimum 1,580 hours in Reșita in 1980. The highest average value was recorded in July or August and the minimum in December , less often in January. The most hours of sunlight were recorded in July 1963, in Timișoara: 290 hours.

The factors that determine the frequency and speed of the wind are related to the general circulation of the atmosphere, to which are added the influences of the local circulation. The magnitude of baric and thermal gradients related to baric formations, convection, all influence wind speed. Westerly winds predominate, with particularities determined by the above factors. Atmospheric calm prevailed, in percentages varying between 21.5% in Sânnicolau Mare and 66% in Vărădia de Mureș.

Romania's relief configuration and Mediterranean influences mean that the average number of days with blizzards was very low. In 30 years, the average number of days per year was less than 1.

In the mountain area, the average started from 9.8 days and exceeded this figure in Țarcu, by 21 days.

The most snow days were recorded in the cold season of the year and in the mountain areas, where they occurred in all seasons of the year, with the highest frequency in January and December.

In the southwestern area of Romania, the annual average number of days with frost varied between 60 and 90 days in the hill and plain areas and fell below 50 days in the mountains.

On average, the first frosty days appeared in the plains in the second half of October and the last frosty days in mid-April. For example, in Timișoara, the first fog was reported on average around October 16 and the last on April 16.

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THE EFFECT OF ADDITIONAL OXYGENATION AND LED LIGHTING ON THE GROWTH OF LUGANO AND CARMESI LETTUCE VARIETIES CULTIVATED IN THE NFT SYSTEM

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Abstract

The oxygen concentration in the nutrient solution is extremely important for the health and development of plants, including lettuce. Lettuce plant roots perform respiration, a process through which they absorb oxygen and release carbon dioxide. This metabolic process is essential for providing the energy necessary for growth and optimal plant functioning. An adequate oxygen concentration in the nutrient solution is crucial for the roots to efficiently carry out this process. Insufficient oxygen concentration can lead to inefficient nutrient absorption, thus affecting plant growth and development. The purpose of this paper is to investigate the impact of different lettuce varieties and cultivation technologies on the average plant mass, length, and average root volume. It examines how various growth conditions, such as additional oxygenation and LED lighting, influence the development of lettuce plants, especially the Lugano and Carmesi varieties. By evaluating these factors, the paper aims to provide relevant information for optimizing agricultural practices in lettuce cultivation, with the goal of maximizing production and crop quality.

***Key words**: Oxygen, roots, nutrients, respiration, development.*

INTRODUCTION

Lettuce represents the most cultivated plant in the category of greens in hydroponic systems (Ohse et al., 2009; Ryder, 1999). In these systems, the lifecycle duration of lettuce is shorter compared to traditional cultivation systems. The NFT (Nutrient Film Technique) cultivation system is ideal for growing lettuce (*Lactuca sativa* L.), allowing for even eight harvests within a calendar year (Fussy & Papenbrock, 2022). In the context of hydroponic lettuce crops, it is recommended that nutrients be presented in the nutrient solution at the following concentrations: 200 mg/L (ppm) of nitrogen (N), 50 mg/L (ppm) of phosphorus (P), 300 mg/L (ppm) of potassium (K), 200 mg/L (ppm) of calcium (Ca), and 65 mg/L (ppm) of magnesium (Mg) (Schon, 1992).

Within unconventional cultivation systems, it is crucial that the nutrient solution contains all the essential nutrients for the growth of lettuce plants. A lack of these elements can lead to the emergence of physiological problems which, ultimately, affect the quality of the lettuce plants (Henry et al., 2019). Lettuce plants develop optimally when the dissolved oxygen content in the nutrient solution reaches at least 6 ppm. No significant variations were observed in terms of the appearance of the roots or the vegetative mass of the lettuce at any of the tested oxygen concentrations, ranging between 2.1 and 16.8 mg/L (Goto et al., 1996). Following the investigation on the impact of oxygen concentrations on hydroponic lettuce cultivation, Goto et al. (1996) concluded that a concentration equal to or less than 2.1 mg/L of dissolved oxygen is necessary to ensure adequate plant development.

The introduction of additional oxygen into hydroponic (NFT) lettuce crops positively influenced the harvest (Kratky, 2005). It was found that a flow rate of 1.5 L/min of the nutrient solution led to an increase in the vegetative mass of lettuce plants compared to flow rates of 0.75 L/min (Al-Tawaha et al., 2018).
The respiration of plant roots is influenced by several factors, including high temperature and salinity levels (Zinnen, 1988). In hydroponic cultivation systems, high temperatures of the nutrient solution and prolonged periods of heat have a negative effect on plant growth (Al-Rawahy et al., 2023; Micu et al., 2022).
Oxygen deficiency near the roots is a critical factor (Fagerstedt et al., 2023). The absence or insufficiency of oxygen can negatively influence the development of the root system and can even lead to its deterioration, which may cause plant desiccation (Boru et al., 2003).
The importance of the presence of oxygen in the root zone of plants throughout all stages of development has highlighted oxyfertigation as an accelerator of the effects of resources in a natural manner (Moreno et al., 2020). In hydroponic cultures, it is crucial to have an elevated level of dissolved oxygen (DO) to support healthy root respiration (Chun & Takakura, 1994). The importance of oxygen absorption using peroxides or peracetic acid in nutrient solutions to enhance the fresh mass in arugula (*Eruca sativa* Mill.) culture developed in a floating system was observed (Carrasco et al., 2011). Research conducted on lettuce grown in a hydroponic system demonstrated that oxygen supersaturation stimulates plant growth without affecting the chlorophyll in the leaves (Kurashina, 2019). Adjusting the water flow rate at the time of harvest can improve crop performance (Baiyin et al., 2021). However, following the results obtained, there is no justification for using a nutrient solution flow rate greater than 2.5 L/min (Stoica et al., 2022). A low oxygen content in the root zone can create favorable conditions for the onset of plant diseases (Cherif et al., 1997) and may promote the infestation of the root system with pathogens, such as *Phytophthora infestans* (Lal et al., 2018). LED lighting allows for the adjustment of the light spectrum to suit the needs of the plant. Red (R) and blue (B) lights are beneficial for photosynthesis. Various studies have investigated the effect of the R and B spectral components on the physiological, biochemical aspects, and resource use efficiency in lettuce plants (Draghici et al., 2013; Drăghici and Pele, 2013; Panter et al., 2014; Panter et al., 2016). Based on research conducted with red and blue light (RB) spectra at levels of 0.5, 1.2, 3, and 4, provided by LED lamps, compared to reference light from fluorescent lamps (with RB = 1) in six experimental variants, under controlled conditions (with PPFD = 215 μmol m^{-2} s^{-1}) and a 16-hour day length, it was found that LED lighting led to a 1.6 times increase in biological yield and a 2.8 times improvement in energy consumption efficiency compared to fluorescent lamps (Pennisi et al., 2019).

MATERIALS AND METHODS

The research was conducted at the University of Agronomic Sciences and Veterinary Medicine in Bucharest, in the greenhouse block of the Research Center for the Study of the Quality of Agri-Food Products. The lettuce varieties used in the experiment were Lugano and Carmesi, with the seeds having a quality assurance certificate for the biological material (Figure 1).

Figure 1. The Lugano and Carmesi varieties

They were cultivated in an NFT (Nutrient Film Technology) system, during the period January 19 - February 20, 2024. Additional oxygenation of the nutrient solution was achieved using a SERA AIR 550 R PLUS type pump for oxygen enrichment with a low electricity consumption of 8W, with an air flow rate of 9.2 l/min and 55 l/h (Figure 2).
The LED lighting system with specific wavelengths and photoperiod used in the experiment has the following characteristics: power 100W, water resistance rate IP67,

frequency 50 Hz/60 Hz, input voltage: AC 220V, wavelength Full Spectrum 380-840 nm.
The conducted experiment was of a 2 x 2 type with three repetitions. Factor A was represented by the two lettuce varieties Lugano and Carmesi, while Factor B represented the cultivation technology with the following gradations: b1-natural oxygenation, b2-additional oxygenation, b3- additional oxygenation plus LED Lighting (Table 1). Oxygen from other sources was not used in the experiment.

Table 1. Experimental Variants

Factor A	Factor B		
a_1 Lugano	b_1 natural oxygenation	b_2 additional oxygenation	b_3 additional oxygenation plus LED Lighting
a_2 Carmesi			

Planting in the NFT system was carried out on January 19, 2024. Climatic factors, such as light, atmospheric humidity, and temperature, as well as the temperature of the nutrient solution, were monitored throughout the entire vegetation cycle.
Observations and measurements dynamically focused on the evolution of the plants in terms of diameter growth and the formation of leaf numbers. After 30 days of planting in the NFT system, the plants were harvested, and the tested variants were weighed to determine their mass, while the length and volume of the roots were measured using an EPSON Flatbed Expression 11000X Scanner.

Figure 2. SERA AIR 550 R PLUS pump for oxygen enrichment

RESULTS AND DISCUSSIONS

The influence of lettuce varieties on the production of average mass (g) showed that the Lugano variety yielded 100.83 g, while the average mass of the Carmesi variety plants recorded a weight of 115.87 g. The difference in average mass between the two lettuce varieties was 15.04 g and is not statistically significant (Table 2).

Table 2. The influence of lettuce varieties on the production of average mass (g)

Factor A	Average Mass (g))	Difference from Ct	Significance
a_1 Lugano	100.83	Ct	
a_2 Carmesi	115.87	15.04	FS

DL 5% 19.4 DL 1% 27.2 DL 0.1% 38.4

The effect of different cultivation technologies on the average mass of lettuce plants showed that in the case of natural oxygenation (b1), the average mass recorded was 82.02 g. The additional oxygenation variant (b2) resulted in an increase in production of 35.59 g compared to natural oxygenation (b1), statistically ensured as being highly significant.
Additional oxygenation plus LED lighting (b3) recorded a highly significant increase in production compared to natural oxygenation (b1), specifically 43.39 g (Table 3).

Table 3. The effect of different cultivation technologies on the average mass of lettuce plants

Factor B	Average Mass (g)	Difference from Ct	Significance
b_1 natural oxygenation	82.02	Ct	
b_2 additional oxygenation	117.61	35.59	***
b_3 additional oxygenation plus LED Light	125.41	43.39	***

DL 5% - 15.8
DL 1% - 22.2
DL 0.1% - 31.4

The impact of cultivation technologies on the average mass of Lugano and Carmesi lettuce plants showed that for the Lugano lettuce variety (a1) with natural oxygenation (b1), an average plant mass of 56.57 g was obtained. The additional oxygenation variant (b2) led to a highly significant increase in the average mass with a difference of 50.91 g compared to natural oxygenation, specifically 56.67 g. The variant with additional oxygenation plus LED lighting (b3) resulted in an even greater increase in the average mass to 124.57 g, with a difference of 68.01 g compared to natural oxygenation (b1). For the Carmesi variety (a2), natural oxygenation (b1) led to an average plant mass of 110.65 g. Additional oxygenation (b2) led to a highly significant increase in the average mass to 121.35 g, with a difference of 64.78 g compared to natural oxygenation (b1). The variant with additional oxygenation plus LED lighting (b3) resulted in an even greater increase in the average mass to 129.48 g, with a difference of 72.91 g compared to natural oxygenation (b1). These results indicate that the interaction between the lettuce variety and cultivation technology has a highly significant impact on the average mass of lettuce plants. Specifically, the addition of supplementary oxygenation and LED lighting is beneficial for increasing the average mass in both varieties, Lugano and Carmesi, leading to highly significant production increases (Table 4; Figure 3).

Table 4. The impact of cultivation technologies on the average mass of Lugano and Carmesi lettuce plants

Factor B	Factor A	Average Mass (g)	Diffe-rence from Ct	Signi-ficance
b_1 natural oxygenation	a_1 Lugano	56.57	Ct	
b_2 additional oxygenation	a_1 Lugano	107.47	50.91	***
b_3 additional oxygenation plus LED Light	a_1 Lugano	124.57	68.01	***
b_1 natural oxygenation	a_2 Carmesi	110.65	54.09	***
b_2 additional oxygenation	a_2 Carmesi	121.35	64.78	***
b_3 additional oxygenation plus LED Light	a_2 Carmesi	129.48	72.91	***

DL 5% - 15.8
DL 1% - 22.2
DL 0.1% - 31.4

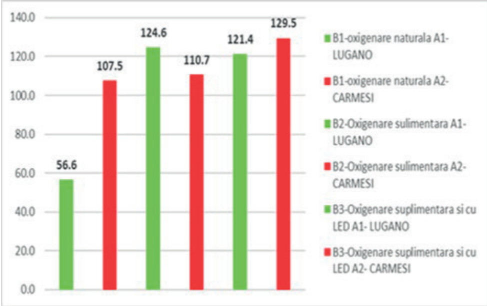

Figure 3. The impact of cultivation technologies on the average mass of Lugano and Carmesi lettuce plants

Root scanning was performed with an EPSON Flatbed Scanner Expression 11000X. For the Lugano lettuce variety (a1), the average root length was 34.47 cm, and for Carmesi, it was 35.51 cm (Figure 4).

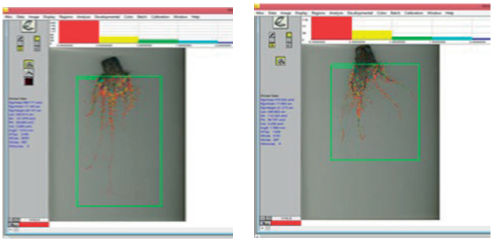

Figure 4. Root scanning of Lugano and Carmesi

The difference in root length between the two varieties is 1.04 cm, not statistically significant (Table 5).

Table 5. Morphometric analysis of root length for Lugano and Carmesi lettuce varieties

Factor A	Average Root Length (cm)	Difference from Ct	Significance
a_1Lugano	34.47	Ct	
a_2Carmesi	35.51	1.07	-

DL 5% - 8.7
DL 1% - 12.2
DL 0.1% - 17.2

Analyzing the morphometric results of the average root length of Lugano and Carmesi lettuce under different cultivation technologies, it was found that there are no significant differences depending on the cultivation technology used. The technology with additional oxygenation (b2) led to an increase in the average root length, with a difference of 3.92 cm compared to natural oxygenation (b1). The introduction of LED lighting along with additional oxygenation (b3) resulted in a difference of 1.85 cm compared to natural oxygenation (b1), but smaller than that observed in the case of additional oxygenation (b2) (Table 6).

Table 6. Morphometric analysis of the average root length of lettuce under different cultivation technologies

Factor B	Average Root Length (cm)	Difference from Ct	Significance
b_1 natural oxygenation	33.07	Ct	
b_2 additional oxygenation	36.98	3.92	-
b_3 additional oxygenation plus LED Light	34.92	1.85	-

DL 5% - 7.1
DL 1% - 9.9
DL 0.1% - 14.0

Analyzing the impact of cultivation technologies on the average root length in the case of lettuce varieties, it is observed that for the Lugano lettuce variety, additional oxygenation (b2) led to a significant increase in the average root length by 5.07 cm compared to natural oxygenation (b1). The introduction of LED lighting along with additional oxygenation (b3) resulted in an even greater increase in the average root length, with a difference of 8.00 cm compared to natural oxygenation (b1). For the Carmesi lettuce variety, natural oxygenation (b1) had an average root length of 35.43 cm, while additional oxygenation (b2) and additional oxygenation plus LED lighting (b3) showed an average root length smaller than natural oxygenation, with differences of 3.80 cm and 4.97 cm, respectively. These results indicate that the interaction between cultivation technologies and the cultivated variety can have a significant impact on the average root length.

Additional oxygenation appears to have a positive effect on root length in the case of the Lugano variety, while for the Carmesi variety, this technology may lead to a decrease in root length. The introduction of LED lighting appears to increase root length in both varieties, but to a greater extent in the case of the Lugano variety. It is important to consider these findings in the development and implementation of agricultural practices to maximize the production and quality of lettuce crops (Table 7; Figure 5).

Table 7. The impact of cultivation technologies on the average root length of Lugano and Carmesi lettuce varieties

Factor B	Factor A	Average Root Length (cm)	Difference from Ct	Significance
b_1 natural oxygenation	a_1 Lugano	30.53	Ct	
b_2 additional oxygenation	a_1 Lugano	35.60	5.07	***
b_3 additional oxygenation plus LED Light	a_1 Lugano	38.53	8.00	***
b_1 natural oxygenation	a_2 Carmesi	35.43	4.90	***
b_2 additional oxygenation	a_2 Carmesi	34.33	3.80	**
b_3 additional oxygenation plus LED Light	a_2 Carmesi	35.50	4.97	***

DL 5% - 2.49
DL 1% - 3.42
DL 0.1% - 4.68

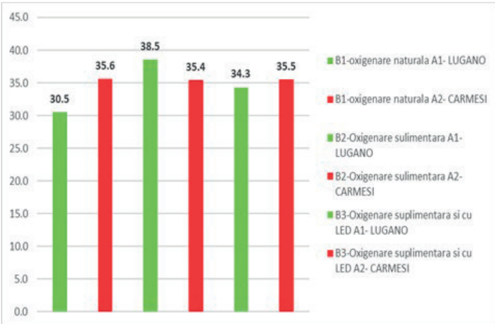

Figure 5. The impact of cultivation technologies on the average root length of Lugano and Carmesi lettuce varieties

The average root volume of the two lettuce varieties was also determined by scanning, with the following results: for the Lugano lettuce variety, the average root volume was 8.62 cm^3, while for the Carmesi lettuce variety, the average volume was 10.06 cm^3.

We observe a statistically insignificant difference between the two varieties, with an increase of 1.43 cm^3 in the average root volume for the Carmesi variety compared to Lugano (Table 8).

Table 8. Analysis of the average root volume of Lugano and Carmesi lettuce

Factor A	Average Root Volume (cm^3)	Difference from Ct	Significance
a_1Lugano	8.62	Ct	
a_2Carmesi	10.06	1.43	-

DL 5% - 1.82
DL 1% - 2.54
DL 0.1% - 3.60

Analyzing the average root volume for the two lettuce varieties, it is evident that with the natural oxygenation technology (b1), the average root volume was 7.25 cm^3. In the case of additional oxygenation (b2), the average root volume was 9.93 cm^3, with a significantly distinct difference of 2.68 cm^3 compared to the technology with natural oxygenation. In the variant with additional oxygenation plus LED light (b3), the average root volume was 10.83 cm^3, with a very significant difference of 3.58 cm^3 compared to technology b1. These results suggest that cultivation technologies involving additional oxygenation, especially when combined with LED light, can contribute to a significant or very significant increase in the average root volume. This is important in the context of growing and developing healthy plants in controlled cultivation systems (Table 9).

Table 9. Analysis of the average root volume of Lugano and Carmesi lettuce under different cultivation technologies

Factorul B	Average Root Volume (cm^3)	Difference from Ct	Significance
b_1 natural oxygenation	7.25	Ct	
b_2 additional oxygenation	9.93	2.68	**
b_3 additional oxygenation plus LED Light	10.83	3.58	***

DL 5% - 1.48
DL 1% - 2.08
DL 0.1% - 2.94

Analyzing the impact of cultivation technologies on the average root volume in the case of lettuce varieties, it is observed that for the Lugano lettuce variety with natural oxygenation (b1), the average root volume was 4.83 cm^3. The technology with additional oxygenation (b2) resulted in a significant increase in the average root volume to 9.67 cm^3, with a difference of 4.83 cm^3 compared to natural oxygenation. In the case of the technology with additional oxygenation plus LED light (b3), it produced a further increase in the average root volume to 10.20 cm^3, with a difference of 5.37 cm^3 compared to natural oxygenation. For the Carmesi lettuce variety in the variant with natural oxygenation (b1), the average root volume is recorded at 9.67 cm^3, the same as in the case of the Lugano variety. The variant with additional oxygenation (b2) resulted in a significant increase in the average root volume to 10.83 cm^3, with a difference of 6.00 cm^3 compared to natural oxygenation. For the technology with additional oxygenation plus LED light (b3), a similar increase in the average root volume was achieved. These results suggest that for both the Lugano and Carmesi lettuce varieties, additional oxygennation and especially its combination with LED lighting have a significant positive effect on the average root volume (Table 10).

Table 10. The impact of cultivation technologies on the root volume of Lugano and Carmesi lettuce varieties

Factor B	Factor A	Average Root Volume (cm^3)	Difference from Ct	Significance
b_1 natural oxygenation	a_1 Lugano	4.83	Ct	
b_2 additional oxygenation	a_1 Lugano	9.67	4.83	***
b_3 additional oxygenation plus LED Light	a_1 Lugano	10.20	5.37	***
b_1 natural oxygenation	a_2 Carmesi	9.67	4.83	***
b_2 additional oxygenation	a_2 Carmesi	10.83	6.00	***
b_3 additional oxygenation plus LED Light	a_2 Carmesi	10.83	6.00	***

DL 5% - 1.80
DL 1% - 2.47
DL 01 % - 3.38

The dissolved oxygen level (DO) was measured using a portable oxygen meter. We conducted measurements of the oxygen level at the inlet and outlet of the culture trough, accompanied by recording the temperature of the nutrient solution (Table 11).

Table 11. Oxygen consumption of plants in the nutrient

Factor B	Factor A	The amount of oxygen in the nutrient solution at the inlet mg/l	The amount of oxygen in the nutrient solution at the outlet mg/l	Inlet solution temperature °C	Outlet solution temperature °C
b_1 natural oxygenation	a_1 Lugano	4.8	3.1	21.6	21.7
b_2 additional oxygenation	a_1 Lugano	6.8	4.8	21.6	21.8
b_3 additional oxygenation plus LED Light	a_1 Lugano	8.2	5.2	21.8	21.8
b_1 natural oxygenation	a_2Carmesi	6	3.4	21.6	21.7
b_2 additional oxygenation	a_2Carmesi	7.7	4.5	21.6	21.8
b_3 additional oxygenation plus LED Light	a_2Carmesi	8.3	5.2	21.8	21.8

CONCLUSIONS

The Carmesi lettuce variety exhibits a higher average plant mass than the Lugano variety; however, the difference in weight is not statistically significant. This suggests that, regarding the average plant mass of lettuce, there is no clear difference between the two varieties. Cultivation technologies involving additional oxygenation, especially when combined with LED lighting, have a very significant positive effect on the average plant mass of lettuce. These technologies lead to significant increases in the average plant mass, both for the Lugano and Carmesi varieties. There are significant differences in the average root length depending on the cultivation technology used. The technology involving additional oxygenation, especially in combination with LED lighting, leads to significant increases in the average root length.

The impact of cultivation technologies varies depending on the lettuce variety. Additional oxygenation appears to have a positive effect on root length in the case of the Lugano variety but may result in a decrease in the case of the Carmesi variety.

Cultivation technologies involving additional oxygenation, especially when combined with LED lighting, significantly contribute to increasing the average volume of roots. This is crucial for the development of healthy plants in controlled cultivation systems. In conclusion, the proper selection and implementation of cultivation technologies can have a significant impact on the mass and development of lettuce plants. Additional oxygenation and LED lighting appear to be effective strategies for improving lettuce yield and quality, although the effects may vary depending on the lettuce variety and specific cultivation context.

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THE INFLUENCE OF TWO TYPES OF EXTRACTS OBTAINED FROM *TAGETES ERECTA* FLOWERS ON RADISH SEEDS

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Abstract

This research paper aimed to evaluate the influence of two types of extracts from the flowers of Tagetes erecta on radish seeds. The extracts were obtained in two organic solvents, ethanol 70% and propylene glycol - PG-50%. Studies were carried out on the phenolic profile, dry matter and antioxidant activity by the DPPH method. The results highlighted that the extract in ethanol had a high concentration only in the total content of flavonoids (6,574 RE mg/mL) compared to the extract in PG (5,111 RE mg/mL) and slightly higher content in polyphenols was found in the case of the PG extract variant. The extract in ethanol 70% registered a higher redox potential (EC50-0.65µl/ml extract). The monitoring of the effect of the extracts was carried out by applying the radish seed germination bioassay. Thereby, the extracts in ethanol showed moderately phytotoxic activity at 0.50% concentration (Gi<80%) and strongly phytotoxic at 1.50% (Gi<50%), and in the case of the extract in PG, they showed moderately phytotoxic activity at 0.50% concentration and a stimulatory effect at 1.50% concentration (Gi>100%).

***Key words**: germination bioassay, Tagetes erecta, biostimulants.*

INTRODUCTION

Recently, the use of products based on biologically active substances in the agricultural and horticultural industry (Rodino et al., 2015), to the detriment of conventional fertilizers and pesticides, has seen a significant increase due to the growing awareness of the population regarding their benefits on plants and the environment (Seufert et al., 2012). Some plants, due to the nature of their composition, show resistance to the attacks of insects and non-beneficial microorganisms. This resistance manifests itself through different processes, including the modification of the biology of pests, a process also called antibiosis (Koch et al., 2016; Fabrick et al., 2020). Besides the plants whose active substances have been exploited to develop products for plant protection, we can mention neem oil, *Chrysanthemum cinerariaefolium*, *Derris elliptica*, *Nicotiana*, and *Ryania* (Kandar, 2015). *Tagetes* species show increasing interest from researchers due to the rich composition of phytochemical compounds with antioxidant properties. *Tagetes erecta* L. is an annual or perennial plant, mostly herbaceous, which is part of the sunflower family (*Asteraceae*), known as an ornamental plant, which presents itself in the form of yellow or intense orange flowers, originally from Mexico (Lokesh et al., 2015). The studies carried out (Buse Dragomir & Nicolae, 2013; Xu et al., 2011) indicate that the *Tagetes erecta* species exhibits nematicidal, fungicidal, repellent and insecticidal activity, and the roots were used in agriculture for the nematicidal activity that persists for a long period.

According to a study carried out by Devika and Justin (2014), regarding the identification of phytochemical compounds by the GC-MS analysis method, on two types of *Tagetes erecta* methanolic extracts (flowers and leaves), it was highlighted that in the *Tagetes erecta* flower extract, were identified 31 phytochemical compounds, and 19 phytochemical compounds were recorded in the extract obtained from the leaves. In leaves of *Tagetes erecta*, were found phenolic compounds with strong antioxidant activities such as caffeic acid, p-coumaric acid and quercetin (Mir et al., 2023). Quercetin (Grajek et al., 2015) shows efficacy against a wide spectrum of bacteria, being effective on both Gram-positive and Gram-negative bacteria. It acts (Wang et al., 2018) by destroying the membrane walls and at the same time inhibits their development. On the other hand, *Tagetes erecta* flowers are rich in flavonoid compounds, including patulitrin, which has a strong antimicrobial activity, especially on pathogens (Mekvimol et al., 2020). In recent years, in agricultural management, more and more products based on natural substances are used for the protection of plants, through the method of cultivation by rotation, this also has applicability in the field of horticulture (Santos et. al., 2015). Thus, this study aims to evaluate the phytostimulant or phytotoxic potential (allelopathic activity of some phytochemical compounds), of two types of extracts obtained from *Tagetes erecta* flowers, in different organic solvents (PG 50% and ethanol 70%), by maceration at room temperature, by applying the germination biotest on radish seeds.

According to Kulbat (2016), some phenolic compounds, such as gallic acid and p-coumaric acid, can exhibit an allelopathic effect on plants. The seed germination bioassay is used, in general, to determine the presence of some phytotoxic or phytostimulant compounds present in the composition of an extract, but also to test its salinity level (Zucconi et al., 1985).

At the same time, were carried out studies to quantify the total compounds of polyphenols and flavonoids, to determine the dry substance from the extracts, as well as the antioxidant activity by the DPPH method.

MATERIALS AND METHODS

Plant material and method of obtaining two extract variants

The dried and ground flowers of the organic culture (*Tagetes erecta*) originating from Bulgaria, were purchased from the trade. To prepare the extracts, the *Tagetes erecta* flowers were washed with purified water and dried in an incubator at a temperature of approximately 40ºC. Were prepared two variants of the extract from marigold flowers (*Tagetes erecta*), in propylene glycol (PG) 50% (DOW Europe Germany, supplier: Medchim) and in ethanol 70% (absolute ethyl alcohol, CHIMREACTIV, Romania) with a ratio of extraction 1:20 (w/v) (Perisoara et al., 2023). The extracts were obtained by maceration in the dark, at room temperature, for 21 days and occasional stirring. After filtering, two extracts of *Tagetes erecta* flowers with fluid consistency of reddish-brown color were obtained (in ethanol 70% and PG 50%). Extracts were stored in the refrigerator (4°C) until future studies and analyses.

Quantitative phytochemical screening

Determination of Phenol Content (TPC)

The method used to quantify the total polyphenol content (TPC) was after the one reported by Sidhu and Saxena (2017). The principle of the method consists of the ability of polyphenols to reduce the Folin-Ciocalteu reagent in the alkaline environment to blue-green carbon oxides with maximum absorption at 765 nm. Thus, 10 µl and respectively 5 µl of the sample were taken, over which was added separately, an amount of methanol required for a total volume of 0.2 mL, followed by the addition of 2.5 mL of Folin-Ciocalteu reagent (diluted with distilled water in a ratio of 1:10 v/v). The mixture was left to incubate for 10 min., at room temperature and protected from light, after which 2 mL of Na_2CO_3 reagent (20%) was added. After incubation at room temperature for 120 min, the absorbance values were recorded at the wavelength of 765 nm. Absorbance was read using a PerkinElmer Lambda 25 UV-VIS spectrophotometer. The concentration of polyphenols was calculated from the calibration curve recorded with caffeic acid in the concentration range of 1-11 μg/mL (R^2 = 0.9974). For the method described, two determinations were performed for each sample and reported as means. The results were expressed in caffeic acid equivalents (CAE) mg/mL test sample extract. All the reagents used (caffeic acid, Folin-Ciocalteu reagent, Na_2CO_3 20% and methanol), were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Determination of Flavonoid Content (TFC)

The colorimetric method based on the property of flavones to react with aluminium chloride in a potassium acetate medium was used to determine the flavone content. Following the reaction, a stable, yellow-chelated complex is formed, whose spectrum shows an absorption maximum in the range of 370-450 nm. The method used to identify TFC was that reported by Cha-Chi et al. (2002), with minor modifications. Rutin was used as a reference reagent to obtain the calibration curve. Thus, amounts of 10 μl and respectively 5 μl of the sample were taken, over which were added 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of 1M potassium acetate and a quantity of distilled water necessary for a total mixture volume of 2.9 mL. After a gentle stirring, the resulting mixture was left to stand for 30 min in the dark, after which the absorbance was read at 415 nm. The absorbance was read using the PerkinElmer Lambda 25 UV-VIS spectrophotometer. The concentration of flavones from the extracts was determined by extrapolation from the calibration curve with rutin, having a dose range of 5-50 μg/mL (R^2 = 0.9996). The samples were processed in duplicate and the results were expressed in rutin equivalents (RE) mg/1 mL sample-extract test. The reagents used rutin aluminium chloride and potassium acetate, were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Evaluation of antiradical capacity

Blank sample: DPPH standard (0.04%) diluted in methanol (2,2-Diphenyl-1-picrylhydrazil) (Sigma-Aldrich Chemie GmbH, Germany) was used. From the obtained standard solution, a sample of 1 mL of the solution was taken, which was mixed with 0.5 mL of methanol (Honeywell, France), then 0.5 mL of distilled water was added, and it was left to rest for 20 min., at room temperature and protected from light. After incubation, the absorbance values at 520 nm were recorded. The extract samples taken in the work were subjected to 1:10 dilution with distilled water. From the diluted extract samples, the following volumes were taken: 1.5 µl, 2 µl, 2.5 µl, 3 µl, 6 µl, 9 µl, 12 µl (in the case of the Fenugreek extract conditioned in PG 50%) and 3 µl, 6 µl, 9 µl, 12 µl, 15 µl (in the case of Fenugreek extract in 70% ethanol) and mixed separately with 500 µl of methanol, 1,000 µl of DPPH reagent (0.04%), over which was added an amount of water required for a total sample volume of 2,000 µl. The samples were left to incubate at room temperature, protected from light, for 20 min. After incubation, the absorbance values were recorded at 520 nm. The absorbance was read using the PerkinElmer Lambda 25 UV-VIS spectrometer equipped with a sample thermostatting system. The whole experiment was worked in triplicate and the results were reported as means. The results are presented as % inhibition as a function of the Log of the applied dose, thus calculating the effective concentration, EC_{50}.

Determination of dry matter

To determine the dry substance from the extracts taken in the study, the gravimetric method SR 7487 - Determination of extractable substances with solvents was used, undergoing slight modifications. This consists of evaluating the amount of product remaining after removing volatile compounds after applying a heat treatment (105°C). Used heat-resistant weighing ampoules, which were dried in an oven at 105°C and cooled in a desiccator at 25°C. This drying-cooling process is carried out in three cycles, the ampoule being weighed at the end of each cycle. At the end, the three weighings are averaged and reported as the final mass (m1) of the weighing vial. An amount of 20 mL of each extract sample is taken and added to the thermostatic water bath, at 95°C, to remove the solvent. Process followed by one hour of drying the two samples in the oven at 105°C. Finally, the samples are cooled in a desiccator for 30 min and weighed, noting the mass m2. The dry matter is determined according to the formula:

(1) [(m2 – m1)] / V x 1000 x 1000 mg/l,

- m1 - mass of the empty capsule, in grams,
- m2 - mass of capsule with residue, in grams,
- V - the volume of the sample (ml).

Seed Germination Bioassay

The purpose of the study was to evaluate the behaviour of *Tagetes erecta* flower extracts in ethanol 70% and propylene glycol 50% on radish seeds. The method used in the radish seed germination study was modified and adapted according to the procedure developed by researchers Miteluț and Popa (2011), Ghayal et al. (2018) and Cristea et al. (2024). The two extracts were studied at concentrations of 0.10%, 0.50%, 1.00% and 1.50%, dilutions were made with distilled water, and the results were reported to the control sample (distilled water); the same dilutions were made for the solvents too (prolylene glycol 50% and ethanol 70%). Before the start of the study, Petri dishes - Corning Petri dishes, with diameter 100 mm and 200 mm, were disinfected with 70% isopropyl alcohol (Contec IPA) and allowed to dry for 24 h. The filter paper - IDL GMBH, blue type, with a diameter of 90 mm, was sterilized in a hood, equipped with a UV lamp for 30 min. on each side. Radish seeds were washed with purified water and dried in an oven at approximately 30°C. After the end of the sterilization process, filter papers were placed in the Petri dishes, on top of which 10 radish seeds of similar size were added and 5 mL of the sample obtained after the dilutions were pipetted. In the case of the control sample, the method is similar, replacing the 5 mL of the sample with 5 mL of distilled water. The plates were deposited in the incubator for 5 days, at a controlled temperature (25°C ± 1°C). The experiment was done in duplicate for all samples. In the end, the germinated seeds were counted - G and the length of the roots - L were measured. The obtained results were processed to determine the germination percentage (GP), the relative seed germination index - RSG, the relative root growth index- RRG and the germination index - Gi, using the following calculation formulas (Yuan et al., 2018):

(2) GP - $\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}$ / x 100%;

(3) RSG - $\frac{\text{Number of germinated seeds in the sample}}{\text{Number of germinated seeds in the control}}$x 100%;

(4) RRG - $\frac{\text{Mean root length of germinated seeds in the sample}}{\text{Mean root length of germinated seeds in the control}}$ x 100%;

(5) Gi = $^{G}/_{G0}$ x $^{L}/_{L0}$ x 100, where,

- G represents the number of germinated seeds on the sample substrate,
- G_0 represents the number of germinated seeds in the control,
- L represents the mean length of plant roots per substrate sample,
- L_0 represents the mean length of the plant roots on the control substrate.

From the specialized data (Emino et al., 2004; Ravindran et al., 2017), the Gi value may indicate a phytostimulant or phytotoxic effect of the extracts on the studied seeds. The germination index values are determined by interpreting and processing the data obtained regarding the relative root growth index and the relative seed germination index. Obtaining a Gi value lower than 50% reveals a strong phytotoxic activity of the extract, a Gi value as close as possible to 0% highlights extreme phytotoxicity of the extract, a Gi value between 50-80% highlights moderate phytotoxic activity, a Gi value between 80-100% reveals an extract with non-phytotoxic activity on the plant, and a Gi value over 100% highlights a product with phytostimulating properties. The study ended when more than 65% of the seeds in the control sample had germinated and/or developed roots at least 20 mm long (EPA, 1996; Alamri et al., 2018).

Statistical analysis

The germination, TPC and TFC studies were performed in duplicate and the antioxidant activity study was performed in triplicate, and the results being reported as mean, standard deviation (±) and relative standard deviation (RSD) were calculated. The statistical analysis was performed using Microsoft Excel 2019. The results were interpreted using the t-test: Paired Two Sample For Means to compare the phytostimulation activity or phytotoxicity of the studied extracts. The *p-value*<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

Quantitative phytochemical screening

In the analysis to determine the content of polyphenols, the Folin-Ciocalteu method was used, and the results were expressed in caffeic acid equivalents (CAE). According to the results obtained (Table 1) regarding the total polyphenol content (TPC) of the two variants of *Tagetes erecta* flower extract, it is highlighted that the extract in PG 50% recorded a slightly higher amount of polyphenols (4,815 CAE mg/ mL), compared to the extract variant in 70% ethanol (4,549 CAE mg/mL). We can say that a better yield regarding the content of polyphenolic compounds was obtained in the case of the PG extract variant. However, some studies reveal the fact that the yield regarding the amount of phytochemical compounds obtained in an extract depends to a large extent on the amount of water used in the extractive mixture. However, in a study carried out by Gong et al. (2011), regarding the chemical composition of some extracts in ethanol (at different concentrations) and water, from *Tagetes erecta*, it was found that the highest concentration of polyphenols was obtained in the case of the extract variant in ethyl alcohol/water (7:3 v/v), 62.33 ± 1.81 GAE/g, and the lowest concentration was obtained in the case of the aqueous extract version, 8.50 ± 0.10 GAE/g. Also, Burlec et al. (2022), following their study on the extracts obtained from *Rudbeckia hirta* and *Tagetes erecta* in different solvents (water, ethanol and ethyl acetate), reported a higher concentration of polyphenols in the case of ethanolic extracts, in both plant species studied. While the extracts in ethyl acetate registered the lowest concentration level in polyphenols, in both types of plants. According to the studies carried out by the researcher Yilmaz et al. (2006), solvent mixtures composed of water and polar alcohols (ethanol, methanol) are more effective regarding the extraction of a higher amount of phytochemical compounds with antioxidant activity, compared to the use of a single solvent. Polyphenols in general exhibit different degrees of polarity, and the selection of a solvent with a polarity as close as possible to what is desired to be extracted, is the key element in ensuring an efficient extraction (Kaczorová et al., 2021). At the same time, polar solvents are effective in extracting a wide range of polyphenols, due to their ability to interact with functional polar groups (Rivas-García et al., 2024).

The content of flavonoids (TFC) was determined by the spectrophotometric method, being in the range of 5,111 RE mg/mL – 6,574 RE mg/mL. The highest value was recorded in the case of the ethanolic extract. Kushwaha et al. (2020), reported a higher concentration of both polyphenols and flavonoids in the methanolic extracts of *Tagetes patula* (of different varieties), compared to the aqueous extracts. Another reference study is the one carried out by Siddhu and Saxena (2017), on *Tagetes erecta* extracts obtained in chloroform, ethyl acetate and methanol, where the highest concentration in flavonoids was observed in the case of the extract variant in methanol (13.43 ± 0.43 mg RE/g extract), and the lowest concentration in flavonoids (7.057 ± 0.66 mg RE/g extract) was seen in the case of the extract variant in chloroform.

Flavonoid compounds are characterized by the property of chelating both metallic copper ions (Cu_{2+}) and iron ions (Fe_{2+}), which contributes to their antioxidant activity. Also, with the help of hydroxyl groups or carbon groups present in the mixture form stable complexes with metal ions (Burlec et al., 2021). As a result of our study, it was observed a higher concentration of flavonoids, compared to the amount of polyphenols, a fact observed in both variants of Marigold (*Tagetes erecta*) extracts studied. Contrary to the results obtained in our study, the specialized literature (Norziah et al., 2015), reveals the fact that polar solvents (methanol and ethanol) can extract phenolic compounds with large molecular size (polyphenols), and aqueous extraction systems better extract flavonoids (phenolic compounds with small molecular size). An observation regarding the differences in the content of polyphenols and flavonoids in the two extracts obtained from *Tagetes erecta* flowers could be given by the polarity of the solvent system used, but also by the variety of phytochemical compounds and the complexity of the composition of the plant (Kowalczyk et al., 2013; Rivas-García et al., 2024).

Table 1. The results of TPC, TFC and antioxidant activity

Sample	TPC (CAE) mg/mL	TFC (RE) mg/mL
Tagetes erecta flower extract in PG 50%	4,815	5,111
Tagetes erecta flower extract in ethanol 70%	4,549	6,574

Evaluation of antiradical capacity

The antioxidant capacity of the two variants of *Tagetes erecta* flower extract was determined by the DPPH spectrophotometric method. The method is characterized by the discolouration of the stable free radical DPPH, by the delocalization of an electronic charge, which manifests itself on the entire surface of a molecule and the measurement of the absorbance at the wavelength of (λ) 520 nm. This is directly proportional to reduced free radicals in the mixture (solution). Antiradical activity is defined by the number of antioxidant substances useful to reduce the initial DPPH concentration by half (50%) and is defined as the effective concentration (EC 50). The results are presented in Table 2, as % of inhibition as a function of the Log of the applied dose, thus also calculating the effective concentration (EC 50). According to the obtained results, the ethanolic extract recorded the most pronounced antioxidant response (EC 50-0.65 µl/mL extract). Ciobanu and his colleagues (2021), highlighted in their study of three variants of propolis, that the most pronounced antiradical activity was obtained in the case of the extract variant with the highest concentration of phenolic compounds present in the mixture. According to the data from the specialized literature (Selma et al., 2014), the extracts that present in their composition a higher concentration of phenolic compounds (polyphenols or flavonoids) will present a higher antioxidant activity. This is also confirmed in our study, where the extract from *Tagetes erecta* flowers in ethanol had a higher concentration of flavonoids, compared to the extract obtained in propylene glycol.

Xu et al. (2015), conducting a study on the antioxidant activity of some *Tagetes erecta* flower extracts obtained at different temperatures (subcritical water extraction SWE), found that the extracts that had in their composition a higher concentration of phenolic compounds showed higher antiradical activity. There are studies in the specialized literature (Leopoldini et al., 2004) where it was highlighted that gallic acid is one of the most active phenolic compounds regarding the transfer of hydrogen atoms and electrons. At the same time Rice-Evans et al. (1996), support these studies by demonstrating the effect of the chemical structure of phenolic compounds on antiradical activity.

Table 2. Antioxidant activity (DPPH) of *Tagetes erecta* flower extracts

Sample	DPPH		
	The regression equation	The regression coefficient	EC 50 µl/ml extract
Tagetes erecta flower extract in PG 50%	y = 74.02x - 10.774	R^2= 0.9755	0.75
Tagetes erecta flower extract in ethanol 70%	y = 74.479x - 10.286	R^2= 0.9828	0.65

Determination of dry matter

Table 3 highlights the results obtained regarding the determination of the dry substance (residue) from the two variants of *Tagetes erecta* extract, where the highest amount of dry matter was obtained in the case of the extract obtained in propylene glycol (25,920 mg/L). If we were to correlate with the results previously obtained, the *Tagetes erecta* flower extract in PG 50%, had a slightly higher amount of TPC, compared to the studied ethanolic extract. But a point that must be taken into account would be the nature of the solvent in which the extraction was done. Propylene glycol has a higher molecular weight, compared to the ethyl alcohol used in the extraction process of the second variant of the extract. A study reported by Perisoara et al. (2023), regarding the determination of the dry matter of three extracts from *Foenum-graecum* seeds in various solvents (ethanol 40%, propylene glycol 50% and ethanol 70%), showed that the extract richest in phenolic compounds (ethanol 40%) has the largest amount of dry matter, followed by the extract in propylene glycol 50% and the one in ethanol 70%. These results are directly proportional to the amount of phenolic compounds, both flavonoids and polyphenols, found in each of the three variants.

Table 3. Results regarding the final residue obtained from the two variants of *Tagetes erecta* extract

Sample	m1 (g)	m2 (g)	The result obtained (mg)	Sample volume (mL)	The final result (mg/L)
Tagetes erecta flower extract in PG 50%	24.5854	25.1038	5,184	20.00	25,920
Tagetes erecta flower extract in ethanol 70%	21.3445	21.7371	3,926	20.00	19,630

Seed Germination Bioassay

The seeds can absorb various substances from the treated environment, be they nutrients or substances with an allelopathic effect, and this is best reflected in the way the roots develop and grow (Ravindran et al., 2017; Baca et al., 1990; Oncel et al., 2000; Araujo & Montero, 2005). The effect on some plants, whether stimulating or phytotoxic, is indicated by the value of the germination index (Gi). This is determined by quantifying the values obtained in the case of the germination index (RSG) and the relative growth of the roots (RRG) (Ravindran et al., 2017). In the current study, the effect of the two variants of *Tagetes erecta* extract (ethanol 70% and PG 50%) at different concentrations on radish seeds was compared, with the solvents used in the extraction process. This was done to reveal the potential stimulating or phytotoxic effect of the obtained extracts.

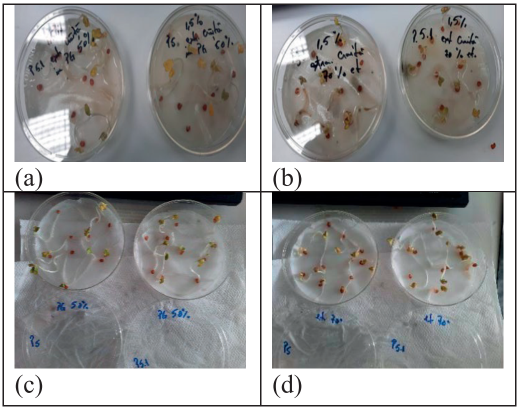

Figure 1. Seedling growth germination bioassay of radish seeds: (a) 1.50% *Tagetes erecta* in PG 50%; (b) 1.50% *Tagetes erecta* in ethanol 70%; (c) 1.50% ethanol 70%; (d) 1.50% propylene glycol 50%

Interpretation of the germination percentage (GP%) was achieved by reporting the number of germinated seeds from the sample/control to the total number of seeds tested (Luo Y. et al., 2017). The germination percentage (GP%), obtained after treating the radish seeds with the two variants of *Tagetes erecta* extract, but also with its solvents, at concentrations of 0.10%, 0.50%, 1.00% and 1.50%, fell between 90-100%. As can be seen from Table 4, the lowest values were observed in the case of *Tagetes erecta* extract in 70% ethanol, at concentrations of 0.10%, and 0.50%. However, in the case of treating radish seeds with the PG 50% solvent, a lower percentage (90%) was observed in the case of the positive control (distilled water). If we were to compare the results obtained in the case of *Tagetes erecta* extract in 70% ethanol with the solvent, it can be observed that in the case of the solvent, the seeds had a germination percentage of 100% at all concentrations studied. Regarding the extract of *Tagetes erecta* in PG 50%, it obtained a germination percentage of 100% at all concentrations compared to the tested solvent. The latter registered GP values (%) below 100% at concentrations of 1.00% and 1.50% (GP - 95%).

Table 4. Germination bioassay results - germination percentage (GP)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	100% ± 5.50 RSD = 57.97	100% ± 0 .00 RSD = 0.00	100% ± 0.00 RSD=0 .00	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00
Tagetes erecta flower extract in ethanol 70%	90% ± 1.40 RSD = 15.71	90% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00	95% ± 0.00 RSD = 0.00	95% ± 0.71 RSD = 7,44
Ethanol 70%	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD= 0.00	100% ± 0.00 RSD =0.00	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00
Propylene glycol 50%	100% ± 0.00 RSD = 0.00	100% ± 0,70 RSD= 7.44	95% ± 0.70 RSD = 7.44	95% ± 0.00 RSD = 0.00	90% ± 0.00 RSD = 0.00

RSG values (%) were obtained by reporting the number of germinated seeds from the extract sample to the number of germinated seeds from the control sample (Cristea et al., 2024). In the case of the results obtained for the relative germination index - RSG, they were: 90-100% for *Tagetes erecta* extract in PG 50%, similar results being observed in the solvent. However, at different concentrations. In the extract, we can observe RSG values of 100% at concentrations of 1.00% and 1.50%. In the case of the solvent, the RSG values decrease directly in proportion to the increase in the dose of the test sample. For the 70% ethanolic extract, the results were between 94.73-105.26%. The highest value was recorded at the concentration of 1.00% extract (statistically insignificant compared to the solvent *(p>0.05)*). The solvent recorded values of 100% at all tested doses. Following the results obtained, we can see that the relative germination index is improved compared to both the solvent and the positive control (distilled water), in both extract variants, being closely related to the concentration of phenolic compounds found in each extract. This was also observed by Perisoara et al. (2022), where they highlighted, following the germination study of *Tagetes erecta* extract in 40% ethanol on radish and cucumber seeds, the fact that the extract had a positive, statistically significant influence on radish and cucumber seeds, compared to the positive control and the solvent, at all tested concentrations.

Table 5. Germination biotest results - relative germination index (RSG)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	95%	90%	100%	100 %	100 ±0.00
Tagetes erecta flower extract in ethanol 70%	94.73 %	94.7%	105.20 %	94.73%	95± 0.71
Ethanol 70%	100%	100%	100%	100%	100± 0.00
Propylene glycol 50%	100%	95%	95%	90%	90± 0.00

The data on the relative growth index of the roots were obtained by reporting the average of the roots developed in the tested extract sample to the average of the length of the roots in the control sample. The values obtained regarding the RRG% index fell within 33.74-103.23%. The highest values were recorded in the case of the ethanolic extract of *Tagetes erecta* (103.70%), at the concentration of 0.10%, followed by the extract in PG (103.23%), at the highest concentration tested (1.50%). After treating the radish seeds with the two extract variants, the RRG index was improved (Table 6), compared to both the control and the solvent. Thus, the *Tagetes erecta* extract in PG compared to the solvent (PG), recorded higher values at all tested doses. This is due to the beneficial effect shown by the phenolic compounds present in the composition of the extract. Regarding the ethanolic extract, compared to the solvent (ethanol 70%), the RRG values were improved only at the concentrations of 0.10% and 1.00%. This could be explained by the presence of a higher concentration of flavonoids in the composition of the extract, showing an allelopathic effect on the plant at the concentrations of 0.50% and 1.50%. Following the germination study conducted by Buse Dragomir & Nicolae (2013), of the extract obtained from the roots of *Tagetes erecta* on the seeds of *Brassica oleracea* (var. *capitata* and var. *botrytis*), tested at different concentrations (2.00%, 5.00%, 10.00 and 20.00%) it was found that the length of the roots decreased significantly compared to the control, directly proportional to the increase in the dose of the tested extract.

Table 6. The results of the germination bioassay - the relative root growth index a (RRG)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	*86.3% (6.95 cm ± 0.33 RSD = 4.88)	86.0% (6.92 cm ± 1.46 RSD = 21.13)	92.10% (7.4 cm ± 0.43 RSD =5.91)	103.2% (8.30 ± 1.57 RSD = 18.98)	**8.0 cm ± 2.41 RSD = 29.97
Tagetes erecta flower extract in ethanol 70%	103.7% (4.9 cm ± 4.05 RSD =82.83)	67.5% (3.19 cm ± 2.71 RSD = 85.11)	94.49% (4.46 cm ± 3,11 RSD = 69,83)	51.64% (2.44 cm ± 0.42 RSD = 17,38)	4.72 cm ± 5.22 RSD = 7.44
Ethanol 70%	88.56% (5.77 cm ± 2.71 RSD = 8.08)	82.9% (5.40 cm ± 1.32 RSD = 24.46)	*77.9% (5.08 cm ± 0.86 RSD = 16.98)	56.63% (3.69 cm ± 2.85 RSD = 0.42 77.41)	6.51 cm ± 0.84 RSD = 12,91
Propylene glycol 50%	74.13% (6.35 cm ± .48 RSD = 7.57)	72.7% (6.23 cm ± 3.59 RSD = 57.65	33.74% (2.89 cm ± 2.04 RSD = 70.71	62.46% (5.35 cm ± 0.93 RSD = 17.44	8.56 cm ± 1.01 RSD = 11.85

*statistically significant compared to control (p>0.05).
**Average root length of germinated seeds expressed in cm

Gi values (%) were obtained by combining the results obtained in the case of RSG and RRG (Sobarzo-Bernal et al., 2021). In Italian legislation, the germination index is included in the list of quality assurance regulations regarding the commercialization of composts (Cesaro et al., 2015). According to the results highlighted in Table 7, the values of Gi (%), fell between 32.05-103.23%. Thus, it can be observed that the extract of *Tagetes erecta* in PG 5% showed a phytostimulant effect (Gi - 103.23%) at the maximum tested concentration (1.50%). At the same time, it has a moderate phytotoxic effect at a concentration of 0.50%

(Gi - 77.47%), registering a Gi value (%) below 80%. Compared to the solvent, Gi values (%) were significantly improved at all doses studied. In the case of *Tagetes erecta* extract in ethanol 70%, a non-phytotoxic effect was observed at 0.10 and 1.00% concentrations, recording values of Gi (%) close to 100% (98.24 and 99.47%, respectively). The ethanolic extract showed a strong phytotoxic effect at 1.50% concentration (Gi - 48.92%). Such an effect was also found in the case of the PG 50% solvent, at a concentration of 1% (Gi - 32.05%), recording a Gi value (%) below 50%. The study reported by Mavi (2014) regarding the treatment of aged seeds of eggplant (*Solanum melongena* L.) with two types of extract obtained from the flowers of *Tagetes erecta* and *Tagetes patula*, to improve the germination rate and the way the seedlings develop, it was observed that the percentage of germination was statistically improved compared to the control and the Hydropriming method, for both types of extract (GP - 82 and 80%, respectively). From the obtained data, a close connection can be observed between the composition of phenolic compounds, the antioxidant activity and the results obtained in terms of the germination study of extracts from radish seeds. The allelopathic effect can be given by the high concentration of phenolic compounds and most of them are reported as phytotoxic compounds (El-Gawad et al., 2015; Elshamy et al., 2019). According to the data in the literature (Li et al., 2010; Anwar et al., 2021), the phytotoxic effect manifests itself through different processes, namely, by the inhibition of respiration, the absorption capacity of nutrients, but at the same time, it can affect the process and activities of the enzyme-linked receptors.

The ethanolic extract of *Tagetes erecta*, presented a higher concentration of flavonoids, and this was found in the manifestation of a more pronounced antioxidant activity, but also a strong phytotoxic effect. Similar results were observed by Santos et al. (2015) studying the phytotoxic activity of two *Tagetes* species (*Tagetes erecta* and *Tagetes patula*), reporting a higher antioxidant activity at the maximum tested concentration (4,000 $\mu g \cdot mL^{-1}$). The ability to interfere with DPPH radicals, but also the power to reduce Fe_2 ions can be related to the high content of phenolic compounds (flavonoids) (Siriamornpun et al., 2012). Some studies (Huckelhoven & Kogel 2003) have shown that certain phytochemical compounds with antioxidant activity can intervene in the process of plant development and germination. Instead, the extract of *Tagetes erecta* in PG 50%, having a more balanced content of phenolic compounds, led to the manifestation of a phytostimulating effect on the radish seeds. For both extracts, these effects were observed at the highest concentrations tested.

Table 7. Germination biotest results - Germination index Gi

Sample	% sample			
	0.10	0.50	1.00	1.50
Tagetes erecta flower extract in PG 50%	82.06	77.47	92.10	103.23
Tagetes erecta flower extract in ethanol 70%	98.24	63.95	99.47	48.92
Ethanol 70%	88.56	82.96	77.97	56.63
Propylene glycol 50%	74.13	69.10	32.05	56.21

semnificativ statistitic comparativ cu martor ($p>0.05$*).
**Average root length of germinated seeds expressed in cm.

CONCLUSIONS

Two variants of *Tagetes erecta* flower extract were obtained using different extraction solvents that are part of the category of concentrated alcohols (ethanol 70% and propylene glycol 50%). These extracts were obtained by maceration, without the intervention of any heat treatment. The main objective regarding the use of these types of solvents and the method of obtaining the extracts was to extract compounds in the highest possible quantity, with potential phytostimulant and antimicrobial (fungicide) effect and not to damage potential extractable compounds. This could be proven by the high content of phenolic compounds (polyphenols and flavonoids) obtained in the two extract variants. At the same time, the work offers valuable information regarding the obtaining in an economical and environmentally friendly way of future natural products based on biologically active substances, intended for the protection and phytostimulation of plants.

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RESEARCH ON THE VARIABILITY OF PHENOLOGICAL CHARACTERISTICS IN GARLIC GENOTYPES AND THE INFLUENCE OF ENVIRONMENTAL FACTORS ON DIFFERENT VEGETATIVE STAGES

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Abstract

The objective of this study was to analyse the variability of phenological characteristics in garlic genotypes over two vegetation years and to assess the influence of environmental factors on different growth stages across 36 genotypes and two garlic varieties. The primary phenological development stages were examined according to BBCH standards, considering the specific environmental factors of the experimental location (44°21'55"N, 23°48'18"E). For autumn varieties, the vegetative period of garlic genotypes was longer during the 2022/2023 season (253.79 days) compared to 2021/2022 (224.99 days). Conversely, in spring varieties, the vegetative period was shorter in 2023 (108.99 days) than in 2022 (130.5 days). This reduction was attributed to abundant precipitation, influencing the growth and development of the crop and reducing the plant's lifespan. The results underscore the significant impact of environmental factors, particularly temperature and precipitation, on the developmental stages of garlic plants.

Key words*: Allium sativum L., climatic impact, crop phenology.*

INTRODUCTION

The phenology of plants represents the recurrent annual sequence of developmental stages, crucial for the functioning of plants and ecosystems (Piao et al., 2019). This sequence undergoes alterations in response to global climate changes, which have the potential to desynchronize ecological interactions and threaten ecosystem functioning (Thackeray et al., 2016). Factors influencing plant growth and development include variations in temperature and soil moisture, nutrient availability, light, and increased CO_2 concentration (Nord & Lynch, 2009). These changes have significant consequences for ecosystem productivity, the carbon cycle, competition, trophic networks, and other ecosystem functions and services (Tang et al., 2016). Field observations provide a method offering direct evidence of phenological changes, accurately recording the timing of phenological events in specific locations and species and providing valuable information on climate change (Piao et al., 2019). Field observation must yield consistent information, even when recorded by different individuals in diverse locations, considering genetic diversity (Popa et al., 2023). In this context, the BBCH system is frequently employed, providing a standardized definition of plant developmental stages (Meier et al., 2009). An extended BBCH scale for garlic, comprising seven main growth stages divided into secondary stages, has been detailed and represented by a code of two or three digits by Lopez-Bellido et al. (2016). Morphological studies, correlated with phenology, have been conducted by other researchers to better understand processes and key traits in the development and growth of garlic plants and their implications for final crop yield simulations (Hsiao et al., 2019) or to analyse phenotypic plasticity and functional responses to water availability (Sanchez-Virosta et al., 2021). Considering these aspects, this work aimed to analyse the variability of phenological characteristics in garlic genotypes over two vegetation years and to examine the influence of environmental factors on different growth stages.

MATERIALS AND METHODS

Biological Material and Research Location
A comprehensive examination involved 36 garlic genotypes (16 autumn varieties: CR14, IZ6, RC12, PV8, SS4, GH2, DB11, CN7, PL1, DG3, GR5, OR10, BR9, M15, DB13, and 20 spring varieties: CR9, CR12, IZ10, RC13, RC14, RC15, SS3, SS19, DB20, CN7, DN8, CZ5, CZ16, C17, PC4, RB11, GL18, BL6, CT1, OT2, FC21), along with 2 approved varieties ('Benone' and 'Cucerdea 80'). Observations were conducted in an experimental plot located in the northern part of Craiova (44°21'55"N, 23°48'18"E). Climatic conditions during the research period (October 2021 - July 2022) were as follows: accumulated precipitation reached 517.6 mm, with an average temperature of 12.7°C; the monthly average minimum temperature was 3.6°C in January 2022, and the monthly average maximum temperature was 26.68°C in July 2022. For the period October 2022 - July 2023, total precipitation accumulated to 717.6 mm, and the average temperature was 13.3°C. Soil characteristics were determined as follows: pH - 6.31, humus content 1.8% (m/m), nitrogen content: nitrites - <0.25 mg/kg, nitrates - 96.6 mg/kg; chemical elements content: P - 496 mg/kg, K - 5410 mg/kg, Mg- 4310 mg/kg, Na - 260 mg/kg, Fe - 23700 mg/kg, Zn - <50 mg/kg. Soil analyses were performed by WESSLING Romania SRL. The cultivation scheme, in rows, had the following distances: 25 cm row spacing, 6-8 cm plant spacing within rows, and a planting depth of 2-3 cm for spring varieties, while for autumn varieties, the planting depth was 5 cm.

Method
The BBCH scale, as described by Meier (2001), was utilized for observations of garlic plant growth stages. Observations commenced from crop establishment (bulb planting), recording each developmental stage: germination phase (00-09), leaf apparatus development (10-19), bulb development (41-48) and senescence (92-99). Additionally, for autumn garlic genotypes developing a flowering stem, the flowering initiation stage (51-59) and full bloom (60-69) were recorded. Observations spanned two cultivation years (2021-2022, 2022-2023).

Statistical Analysis
The obtained data were statistically analysed using the Excel data analysis program.

RESULTS AND DISCUSSIONS

Substantial disparities in environmental conditions have been observed, encompassing average monthly temperatures, the seasonal mean temperature, monthly precipitation averages, total precipitation, and their distribution. The data delineated in Table 1 pertains specifically to the temperature factor, revealing an upward trend. In the cultivation season of 2022-2023, the monthly temperature mean surpassed the previous season by +0.60°C. Notably, the lowest value for monthly mean temperatures occurred in the inaugural cultivation season, with January recording 3.66°C. In contrast, the minimal mean value for December 2022, within the 2022-2023 season, was 4.6°C.

Table 1. Average monthly temperatures (°C) for the period October 2021 - July 2023

Period/ Average temperature (°C)			
October 2021	11.91°C	October 2022	15.58°C
November 2021	9.26°C	November 2022	11.21°C
December 2021	4.31°C	December 2022	4.6°C
January 2022	3.66°C	January 2023	6.03°C
February 2022	6.89°C	February 2023	5.35°C
March 2022	6.85°C	March 2023	10.7°C
April 2022	13.3°C	April 2023	12.44°C
May 2022	19.75°C	May 2023	17.69°C
June 2022	24.46°C	June 2023	22.2°C
July 2022	26.68°C	July 2023	27.25°C
Mean	12.70°C	Mean	13.30°C

For spring garlic varieties, the cultivation season spanning from March to July is notably shorter compared to that of autumn varieties. Within this timeframe, considering only the March to July interval, the monthly temperature mean was 18.20°C for the March - July 2022 season and 18.05°C for the March - July 2023 season, showcasing a marginal difference of a mere 0.15°C between the two seasons. Substantial distinctions are identified in October (3.67°C), November (1.95°C), January (2.37°C), and March (3.85°C). Table 2 delineates the monthly and total precipitation quantities for the two cultivation seasons. Data analysis reveals a heightened precipitation amount for the period October 2022 - July 2023, recording 717.5 mm, surpassing the October 2021 - July 2022 period by 199.9 mm (517.6 mm). Concerning the distribution of precipitation across calendar months, no consistent pattern is discerned, with each month exhibiting diverse tendencies annually. In the initial three months of 2023, precipitation amounts were notably higher compared to 2022. Moreover, the concluding months of June and July in the growing season exceeded the values of the corresponding months in the previous year (June 2022 - 63 mm, June 2023 - 111.2 mm; July 2022 - 41 mm, July 2023 - 67.6 mm). Regarding spring varieties, the initial cultivation season (2022) accumulates a total of 345 mm, representing over half of the total precipitation value recorded for the autumn variety cultivation season (2021-2022). The second growth season registers a total of 310 mm, a value proximate to the previous year. However, the distribution therein significantly differs (March 2022 - 5.8 mm; March 2023 - 95.2 mm; July 2022 - 41 mm; July 2023 - 67.6 mm).

Table 2. Amount of monthly precipitation (mm) for the period October 2021 - July 2023

Period/Monthly precipitation (mm)			
October 2021	44.6	October 2022	27.0
November 2021	56.4	November 2022	93.2
December 2021	35.0	December 2022	110.0
January 2022	76.2	January 2023	76.2
February 2022	77.8	February 2023	100.6
March 2022	5.8	March 2023	95.2
April 2022	117.8	April 2023	0.2
May 2022	117.4	May 2023	36.3
June 2022	63.0	June 2023	111.2
July 2022	41.0	July 2023	67.6
Total	517.6	Total	717.5

Table 3 illustrates the progression of phenological stages for autumn garlic genotypes across two cultivation seasons, highlighting variations arising from distinct environmental conditions. In the first year of the study (2021-2022), the initial phenological stage, germination, a period characterized from planting to the emergence of the first true leaf, averaged 30.75 days, with a range between 14 and 69 days, manifesting a 55 day difference among genotypes. In the year 2022/2023, the germination period was shorter than the preceding year, recording an average duration of 26.5 days. The variation limits were narrower compared to the previous year, with a lower limit of 21 days and an upper limit of 35 days, indicating a 14 day difference among genotypes. The duration of the germination period among genotypes was more closely clustered around the mean value for the 2022/2023 season, with the coefficient of variation (CV) at 18.3%, as opposed to the 2021/2022 season when the CV was 52.52%. This difference is attributed to varying environmental factors (Tables 1 and 2). According to specialized literature, factors influencing the garlic clove germination rate encompass planting time, cultivar, environmental factors (temperature), photoperiod, and harvest maturity (Kamenetsky & Okubo, 2012). The second stage, leaf development, spans from the emergence of the first visible true leaf, exceeding 3 cm (11-101 BBCH), to the appearance of the last leaf in the foliage apparatus (19-109 BBCH). For the study period 2021/2022, the leaf development phase lasted an average of 117.66 days. The statistically calculated minimum number of days was 72, while the maximum was 148. In the following growing season, 2022/2023, the vegetation period increased by approximately 20 days compared to the previous year, reaching 137.93 days. Regarding the range limits, the values were much closer to each other, with the minimum limit at 133 days and the maximum at 147 days. The coefficient of variation showed values of 22.03% in the 2021/2022 season and 3.93% in the 2022/2023 season. The onset of bulb formation is considered when the basal leaf begins to thicken or expand (41-401 BBCH). This phase is primarily influenced by genetic and environmental factors, especially photoperiod and increasing temperatures, correlated with the plant's phenological stage (Atif et al., 2019). The end of this phenological stage is recorded when growth is complete, leaves are dry, and bulb dormancy begins (49-409 BBCH). In the 2021/2022 growing season, this phase lasted 61.2 days, with variation limits between 54 and 69 days. In the second year, 2022/2023, the data were approximately similar, with values varying very little compared to the year 2021/2022. The average period was 67.93 days, the range limits were 56 to 77 days, and the coefficient of variation was 9.80%. For genotypes with tall stems, exhibiting "hard neck," which develop a flowering stalk, the flowering phase (51-501 BBCH) occurs simultaneously with the bulb development phase, concluding with the end of flowering (69-609 BBCH). This phenological stage coincides with the final stage of bulb formation and involves the appearance of the flowering stalk, observed only in some autumn garlic genotypes. The phenomenon is influenced by the storage conditions of garlic bulbs and the duration of the photoperiod. Generally, it is considered that low temperatures and a long photoperiod favour the appearance of flowering stalks (Kamenetsky et al., 2004). Mathew et al. (2011) suggest that garlic florescence can be promoted through exposure to appropriate environmental stimuli during storage before planting and sprouting until subsequent growth stages. Regarding autumn garlic genotypes, specialized literature indicates three types: genotypes that do not produce flowering stems ("soft neck," *A. sativum* L. var. *sativum*), genotypes that produce flowering stems, form flowers, and sterile seeds (Kashmiri garlic - *A. schoenoprasum* L.), and genotypes that develop flowering stems and form aerial bulbils, such as Rocambole garlic ("hard neck" - *A. sativum* var. *ophioscorodon*). In the conditions of the 2021/2022 growing season, the flowering phase lasted an average of 53.73 days. The range limits for this phase were between 42 and 69 days, varying with the genotype. In the second growing season (2022/2023), the flowering period significantly decreased, reaching an average duration of 38.6 days. The range limits for this year were between 32 and 45 days.

The physiological maturity of plants represents the necessary stage for further development or final maturity, aiming at the consumption, processing, or multiplication of garlic (Galgaye, 2023). As the ultimate phase, plant senescence initiates from the moment plants begin to discolour (92-902 BBCH) and concludes when they are harvested (99-909 BBCH). For the period 2021/2022, plant senescence lasted an average of 14.26 days, with variation limits between 8 and 19 days. For this phenological stage, the coefficient of variation recorded a value of 26.81%, indicating high variability among the genotypes under study. For the period 2022/2023, the duration of the garlic plant senescence phase was 21.43 days, longer compared to the previous year. The variation limits for this period were between 16 and 27 days.

Table 3. Evolution of phenological stages of autumn garlic genotypes

Phenological stages	Statistical indicators	Period	
		2021/2022	2022/2023
0. Sprouting	X±SD	30.75±16.15	26.5±4.85
	Range limits	14-69	21-35
	CV %	52.52	18.30
1. Leaf development	X±SD	117.66±25.92	137.93±5.43
	Range limits	72-148	133-147
	CV %	22.03	3.93
4. Bulbing phase	X±SD	61.2±6.73	67.93±6.65
	Range limits	54-69	56-77
	CV %	10.99	9.80
5. Flowering phase	X±SD	53.73±9.14	38.6±5.45
	Range limits	42-69	32-45
	CV %	17.01	14.14
9. Senescence	X±SD	14.26±3.82	21.43±3.28
	Range limits	8-19	16-27
	CV %	26.81	15.32

X - mean; SD - standard deviation; CV - coefficient of variability (%)

Table 4 provides a statistical analysis of the phenological stages in spring garlic genotypes. It is noteworthy that spring garlic genotypes do not produce flowering stems; hence, the flowering phase is absent in the study of phenological stages. Similar to autumn genotypes, the recording of phenological stages commenced at the establishment of the crop. In the first growing season, the year 2022, the average duration for the initial phase (sprouting) was 20.1 days. The range varied between 10 and 28 days for the same garlic genotypes. In the subsequent year, 2023, the germination period was shorter compared to the previous year, with an average duration of 13.65 days. The variation limits were narrower than those in 2022, with a lower limit of 10 days and an upper limit of 17 days. The coefficient of variation showed values of 23.99% in 2022 and 17.13% in the 2023 season. The second stage (leaf development) in 2022 spanned a period of 56.6 days, with a minimum of 36 days and a maximum of 68 days. For the next growing season, 2023, this stage recorded a decrease of approximately 9 days compared to the previous year, namely 47.80 days, likely due to higher temperatures in that year. In terms of variation limits, the values were much closer to each other, with a minimum limit of 40 days and a maximum limit of 60 days. The coefficient of variation values was similar for both years, measuring 12.20% (2022) and 12.22% (2023). In the first year of cultivation (2022), the bulb formation phase lasted for 41 days. The range of variation was between 38 and 44 days, with a coefficient of variation of 3.87%. Similar to the previous stage, in the 2023 growing season, the duration of this phenological phase was shorter compared to 2022. The average period was 35.38 days, with variation limits of 30 to 42 days, and a coefficient of variation of 12.30%. The senescence stage of the plants averaged 12.8 days in 2022, with variation limits between 11 and 16 days. In 2023, the senescence period for garlic plants was 12.2 days. The coefficient of variation values was close, with 2022 having a value of 9.68%, and the subsequent year (2023) registering 12.29%

Table 4. Evolution of phenological stages of spring garlic genotypes

Phenological stages	Statistical indicators	Year	
		2022	2023
0. Sprouting	X±SD	20.1±4.82	13.61±2.33
	Range limits	10-28	10-17
	CV %	23.99	17.13
1. Leaf development	X±SD	56.6±6.90	47.80±5.84
	Range limits	36-68	40-60
	CV %	12.20	12.22
4. Bulbing phase	X±SD	41±1.58	35.38±4.35
	Range limits	38-44	30-42
	CV %	3.87	12.30
9. Senescence	X±SD	12.8±1.23	12.2±1.23
	Range limits	11-16	11-14
	CV %	9.68	12.29

X - mean; SD - standard deviation; CV - coefficient of variability (%)

Regarding the duration of the vegetation period, Figure 1 illustrates the phenological stages' duration for autumn garlic genotypes, from crop establishment to plant senescence, for the two cultivation years 2021/2022 and 2022/2023. Differences between the two cultivation years in terms of the period and duration of these stages are observed. It is noted that during the first cultivation season (2021/2022), the progression through phenological phases occurred much faster compared to the following season (2022/2023). In 2021/2022, the crop was established in the third decade of October to the first decade of November. In this case, the end of the vegetative period took place in the last decade of June to the first decade of July, spanning, on average, 7-7.5 months. In 2023, the end of the vegetative period occurred in the first and second decades of July.

Comparatively analysing the two garlic genotypes' cultivation seasons, the following stand out: (1) A difference regarding the flowering period, specifically the absence or presence of the flowering phenological stage. Genotypes that did not emit a flower stem in both cultivation years were: CR14, IZ6, PV8, SS4, DB11, DB13, BR9. There were genotypes that developed the flowering phenological stage in both the 2021/2022 and 2022/2023 years: RC12, GR5, M15. For the three genotypes (RC12, GR5, M15), even though they developed the phenological stage in both cultivation years, the flowering period was shorter in 2022/2023 compared to 2021/2022: RC12 – 53 days (2021/2022) and 45 days (2022/2023); GR5 – 45 days (2021/2022) and 40 days (2022/2023); M15 – 50 days (2021/2022) and 45 days (2022/2023). (2) In 2023, for genotypes that emitted flower stems, aerial bulbils did not develop as expected, having reduced sizes and not reaching the required maturity state for harvesting. According to the literature, flowering and bulb formation in geophyte plants are energy-intensive processes, and the competition for internal resources limits their ability to fully develop (Michael et al., 2018).

Analysing the two study years, the number of days for phenological stages varied even within the same genotype, depending on the climatic conditions of each year. The number of days for the germination stage ranged from 14 days for the 'IZ6' genotype in 2021/2022 to 69 days for the 'M15' genotype in the same year. For the leaf development stage, the number of days ranged from 72 days for 'M15' (2021/2022) to 148 days for 'RC12' (2021/2022). The bulb development phase had a minimum limit of 46 days for 'Benone' (2021/2022) and a maximum limit of 84 days for 'M15' (2022/2023). The flowering stage ranged from a minimum of 32 days for 'BR9' (2022/2023) to a maximum of 68 days for 'PV8' (2021/2022). The senescence stage varied between 8 days for 'PV8', 'SS4' (2021/2022), and 27 days for 'SS4' (2022/2023).

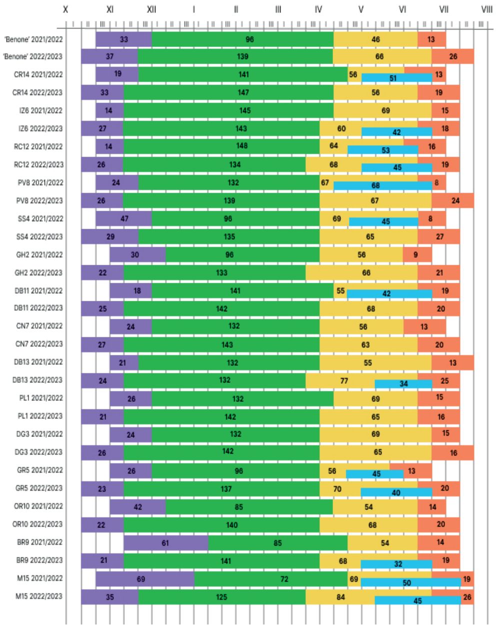

Figure 1. Phenogram of sprouting - senescence of autumn garlic genotype (purple: 00-09; green: 10-19; yellow: 41-48; blue: 51-69; red: 92-99)

Figure 2 illustrates the phenogram of spring garlic genotypes, highlighting all phenological stages for the years 2022 and 2023. A significant decrease in the durations of phenophases is observed for all garlic genotypes in the 2023 growing season. This is caused by a delay in crop establishment due to unfavourable weather conditions. Abundant and frequent precipitation was recorded in the months of February (100.6 mm) and March (95.2 mm), preventing adherence to the planting season, specifically the last decade of March and the first two decades of April. Del Pozo et al. (1994) suggest that the duration of the planting-emergence of shoots and emergence-initiation of bulbs is shorter when sowing is late. The sowing date and varieties are among the critical factors determining garlic yield and quality (El-Zohi et al., 2014). Unfavourable environmental factors affect the vegetation period. Such events were recorded in 2023 in the form of abundant precipitation and hail. Significant amounts of precipitation were recorded in June (111.2 mm) and July (67.6 mm), corresponding to the phenological stage of bulb development. The leaf apparatus of the plants was irreversibly affected, and at this point, the senescence phase of garlic plants occurred in the first and second decades of July. In 2022, garlic plants went through a longer vegetative period, with genotypes planted in the first decade of March, in the first urgency. The end of the vegetation period occurred in the second decade of July.

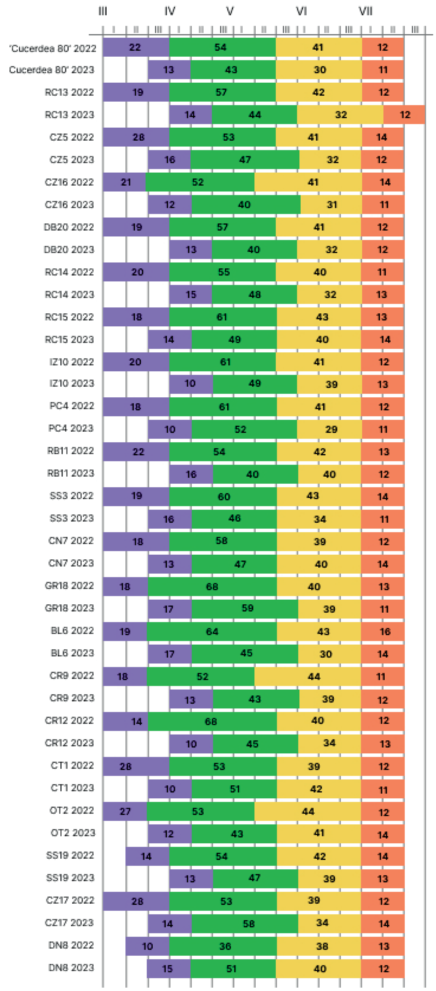

Figure 2. Phenogram of sprouting - senescence of spring garlic genotypes (purple: 00-09; green: 10-19; yellow: 41-48; red: 92-99)

Similar to autumn genotypes, the number of days for phenological stages varied. The number of days for the sprouting stage ranged from 10 days for genotypes 'PC4', 'IZ10', 'CR12,' and 'CT1' in 2023 to 28 days for genotype 'CZ5' in 2022. For the leaf development stage, the number of days ranged from 36 days for 'DN8' (2022) to 68 days for 'GR18' (2022). The bulb development phase had a minimum duration of 29 days for 'PC4' (2023) and a maximum of 44 days for 'CR9' (2022). The senescence stage varied between 11 days for 'Cucerdea 80' (2023) and 16 days for 'BL6' (2022).

CONCLUSIONS

The obtained results emphasize that environmental factors, notably temperature and precipitation, wield a significant influence on the developmental stages of garlic plants.

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EFFECTS OF TREATMENTS WITH BIOPESTICIDE CARBECOL AND FUNECOL ON TOMATO (*SOLANUM LYCOPERSICUM*. L) LATE BLIGHT

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Abstract

Tomato late blight (Phytophtora infestans) is a major disease of tomato (Solanum lycopersicum L.) in many agricultural regions, causing huge losses in vegetable production. A greenhouse experiment was conducted to investigate the efficacy of the new biofungicide Carbecol alone or in combination with treatments of the biofungicide Funecol in the control of late blight in tomato and their impact on crop productivity. Tomato plants were treated four times during the growing period with Carbecol alone or in combination with treatments of Funecol at different concentrations to control late blight of tomato. The experimental results revealed that foliar disease of late blight in tomato was significantly reduced by treatments applied in combinations of Carbecol and Funecol in comparison with untreated plant or Carbecol alone. However, the best results were reached in the variant with integrated application of Carbecol at a rate of 0.4% plus treatments with Funecol at a rate of 0.4%. The treatments had a beneficial effect on the tomato fruit production. The integrated application of Carbecol and Funecol gave the best effect and the yield increased by 16% in comparison with untreated plants.

***Key words**: Biorational products, Carbecol, Funecol, Late blight, tomato.*

INTRODUCTION

Plant diseases are a major threat to agriculture and to food security. Today, organic agriculture is being promoted on a large scale and the market for organic products has grown significantly in many countries as well as in the Republic of Moldova (Fenibo et al., 2021; Suja et al., 2013). It is well documented that plant fungal diseases are the most destructive diseases, where the fungal pathogens attack many valuable crops, causing a significant yield loss (Šunjka & Mechora, 2022). Among them, tomato late blight, caused by the pathogen *Phytophtora infestans*, is the most common foliar disease of tomato, destroying all organs such as leaves, stems, stalks and fruits (Ivanov et al., 2021). Synthetic fungicides have a good efficacy to combat this disease. However, there are growing concerns about the use of synthetic fungicides in terms of environmental and food safety; hence, the need to explore other alternatives that are friendly to farmers, to consumers and to the environment is imperative for the sustainable development of organic vegetable production. In this regard, late blight also is a major constraint for tomato production in the Republic of Moldova. In order to minimize the use of chemical fungicides against late blight, attempts are being made to develop integrated approaches to the use of biorational protection products. According to EU regulations for the organic production of vegetable crops, there is a need to decrease the use of chemical fungicides in crop treatments and to increase the use of alternative biorational protection products (Commission Regulation No 1107/2009; La Torre et al., 2019; Marchand, 2016). Therefore, considering the agronomic and socioeconomic importance of late blight disease and the lack of information on the integrated use of biorational protection products, the aim of this study was to evaluate the efficacy of new biopesticide Carbecol and Funecol applied in different combinations, in controlling late blight disease of tomato.

MATERIALS AND METHODS

To fulfill the purpose of the study, a greenhouse experiment was conducted with tomato plants. A commercial susceptible tomato variety Tolstoy was used in the experiment. The experiment was conducted at the Institute of Genetics, Physiology and Plant Protection, State University of Moldova. The soil used for the experiment was carbonated chernozem.

The trail was designed as a split-plot design with three replications. Treatments with Carbecol and Funecol are shown in Figure 1. Combined applications of Carbecol and Funecol were applied at the following stages of plant development: 1st - at 14 days after transplanting; 2nd - at the intensive growth stage; 3nd - at the flowering stage and the 4th - at the fruit development stage. Carbecol and Funecol were applied separately, in different concentrations.

The treatments were performed by spraying the solution on the tomato leaves. The usual cultural practices were followed during the growing season. In addition to late blight, other diseases, insects and weeds were monitored in the trial by regularly inspecting the plants. The severity of the late blight was determined using the disease severity index (DSI), calculated according to Pandey et al. (Pandey et al., 2003). The index of disease severity was recorded four times at ten days intervals after the last spray. At physiological maturity, tomato fruits from each variety were harvested (10 harvests were made) and weighed separately to determine fruit yield. The experimental data were analyzed using the STATISTIC 7 program.

RESULTS AND DISCUSSIONS

Tomato late blight disease has become one of the major constraints for successful organic cultivation of tomato in the Republic of Moldova. The efficacy of treatments with Carbecol alone or in combination with Funecol against late blight was examined on tomato leaves. The tomato plants were naturally infected with phytopathogen *P. infestans*. The effect of the biorational products Carbecol and Funecol on the percentage of disease severity during the first assessment of the late blight is shown in figure 1. The severity of late blight caused by pathogen *Phytophtora infestans* in the experimental plots was higher than we expected for untreated tomatoes. The experimental data showed that all the tested treatments significantly suppressed the attack of the phytopathogen *P. infestans* on leaves of tomato plants. Experimental results revealed that the disease severity index was minimal in the plots treated with the standard systemic fungicide Cooperon, WP (0.3%). It is known that the Cooperon, WP is a systemic fungicide that has been widely used to control late blight in tomato and other diseases of Solanaceae species caused by oomycetes fungi (Alexandersson et al., 2016; Shashidhara et al., 2009). The data show that there were favorable conditions for the fungus sporulation during vegetation period of tomato plants.

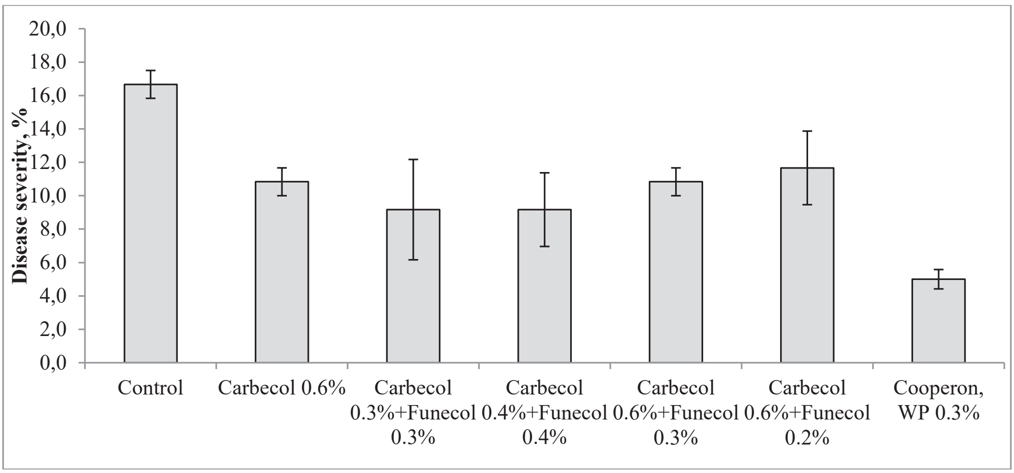

Figure 1. Effect of treatments with Carbecol alone or in combination with Funecol on late blight disease severity of tomato. Vertical bars show mean values and standard error (SE) (The first assessment of the disease index, 24.07.23).

Among the combinations of these two products, the lowest incidence of late blight occurred in the treatment of Carbecol 0.4% combined with Funecol 0.4% treatments (Figure 1). This variant also registered a better effect in controlling late blight in tomato in the next evaluations of disease severity. The results obtained at the second assessment of the disease (Figure 2) showed that all treatments applied had a significant positive effect on reducing the severity of late blight compared to the control. The experimental results showed that all treatments were significantly different from the untreated treatment of control.

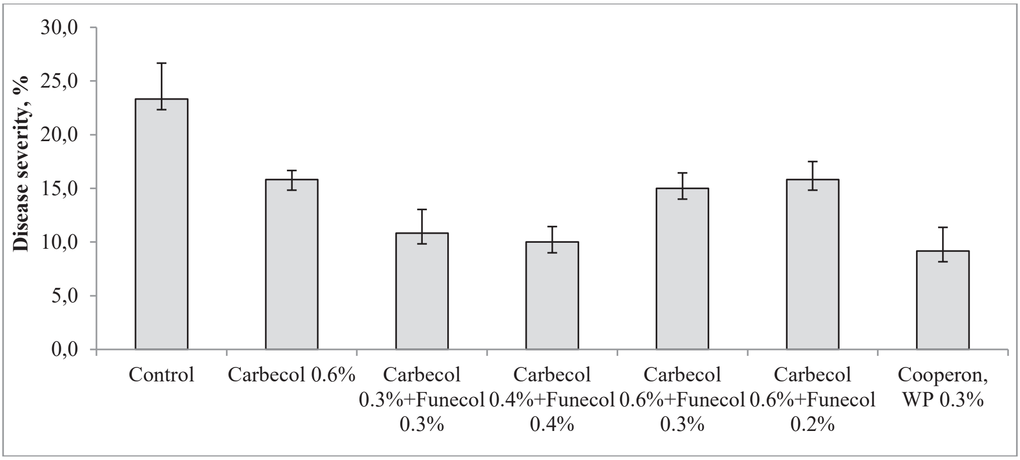

Figure 2. Effect of treatments with Carbecol alone or in combination with Funecol on late blight disease severity. Vertical bars show mean values and standard error (SE) (The second score of the disease index, 7.08.23).

Generally, the greenhouse experiment demonstrated that the application of Carbecol combined with Funecol was more effective in controlling tomato late blight disease than the application of Carbecol alone. It is necessary to note that differences between control plants and treated ones were observed even at the first evaluation of disease severity. Experimental data revealed that upon the first evidence of late blight incidence, the treatment of Carbecol+Funecol at a concentration of 0.4% for each product resulted in a disease severity of 9.2%, whereas in the control treatment, this value was 16.7%. The lowest infection of tomatoes by *P. infestans* was recorded in the variant treated with the chemical fungicide Cooperon, WP with an occurrence of 5%. Therefore, the research data indicated that the treatment of Carbecol 0.4% + Funecol 0.4% offered effective protection against the phytopathogen *P. infestans*. The results of the second score of disease severity of tomato leaves are presented in Figure 2. The best protection (60.7% efficacy) was obtained with the application of chemical fungicide Cooperon, WP, which was slightly better but not significantly different from the variant Carbecol 0.4% + Funecol 0.4% (57.1%). Biofungicide Carbecol applied alone provided the worst protection but significantly different from untreated control, with 32% efficacy (data are not shown). No phytotoxicity was observed on the tomato plants after spray application of the biorational protection products as well as in treatment with standard chemical fungicide. It is necessary to note that the disease severity index in all treatments increased during the vegetation growth of plants. The treatments suppressed the progression of the disease; however, the increase was less pronounced in the chemical treatment of Cooperon, WP. Likewise, a good protection effect was registered in the treatment Carbecol + Funecol at 0.4% concentration of each product in all scores of the index. Nonetheless, the phytopathogen *P. infestans* caused a higher disease severity on the tomato plants in the untreated control. We can suggest that the incidence of late blight, caused by the phytopathogen *P. infestans*, in the treatment with the combined application of biorational products, exhibited the lowest values of disease severity index compared to the treatment with Carbecol alone. Such trend was observed at all evaluation dates for disease severity (Figures 1-4). The analysis of results at the second and third evaluations of disease severity revealed more pronounced differences between variants (Figures 2 and 3). The application of Carbecol alone at a concentration of 0.6% decreased the disease severity from 23.3%, as registered in the control variant, to 15.8%. However, the most significant reduction in disease severity was observed in the variant with the combined use of Carbecol and Funecol (variant 4). Thanks to integrated use of biofungicide Carbecol and Funecol, the late blight severity index was reduced compared to the control variant (untreated plants), indicating a reduction in infection by around 50%. Similarly, the application of Cooperon, WP significantly reduced the attack by pytopathogen *Phytophtora infestans*, resulting in a disease severity of 9.2% (Figure 2).

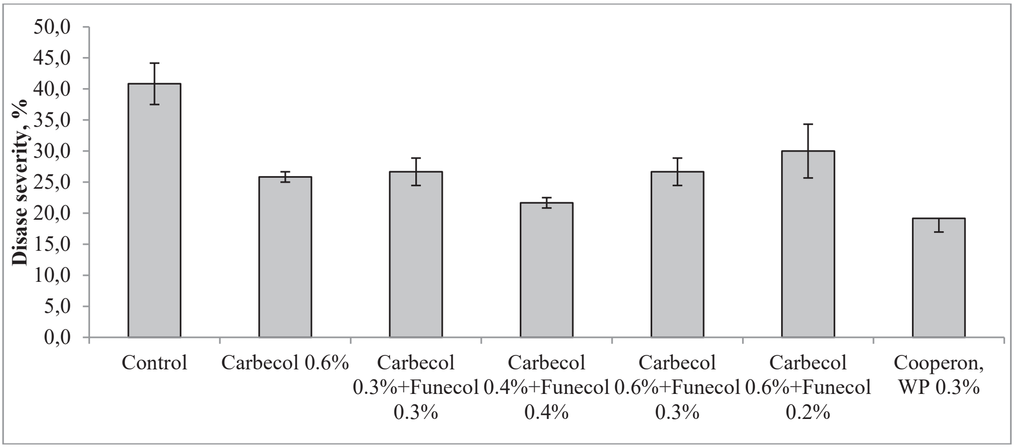

Figure 3. Effect of treatments with Carbecol alone or in combination with Funecol on late blight disease severity. Vertical bars show mean values and standard error (SE) (The third index assessment, 17.08.23).

In general, the lowest incidence of the disease caused by *P. infestans* was observed in tomato plants treated with systemic fungicide Cooperon, WP as well as in the treatment of integrated use of Carbecol and Funecol (variant Carbecol 0.4% + Funecol 0.4%). We stated that there were no significant differences in effectiveness among the biopesticide combination of Carbecol and Funecol and chemical fungicide Cooperon, WP.

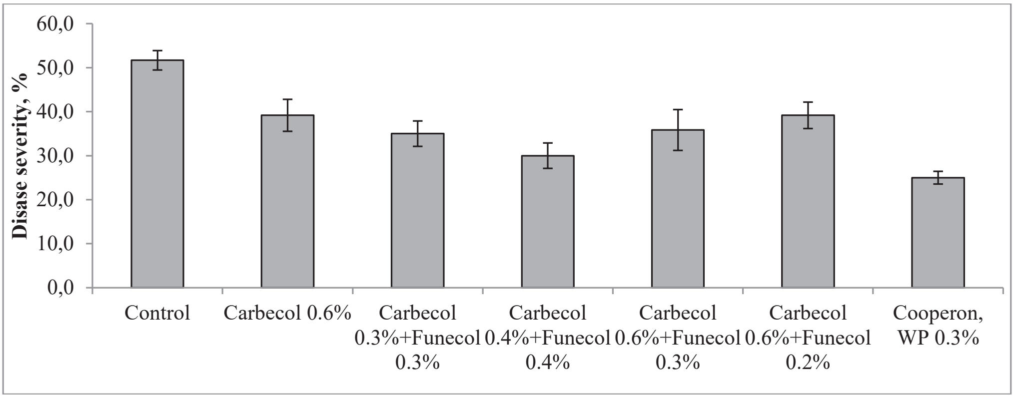

Figure 4. Effect of treatments with Carbecol alone or in combination with Funecol on late blight disease severity. Vertical bars show mean values and standard error (SE) (The fourth assessment index, 27.08.23).

As the growth of plants progressed, the disease incidence of late blight caused by *P. infestans* increased regardless of treatments. Figures 3 and 4 showed the mean percent of disease severity at the third and fourth scores of the index. The treatments with the lowest incidence of late blight at the third evidence of disease index were those of combined application of both biorational products and synthetic fungicide Cooperon, WP (Figure 3). Of course, the highest incidence of late blight was at fourth evidence of the disease index (on the end of august) especially in the untreated control variant. However, the integrated application of these two biorational products reduced significantly the severity of this disease also in august. In the treated plots, the disease progress in the plants was much slower and the severity of disease was much less than in control plots. It was revealed that the treatment of Cooperon, WP was most effective in controlling late blight and was not significantly different from the treatment of biorational products Carbecol 0.4% + Funecol 0.4%. Similar, as in the previously three assessments of the disease, the highest incidence of late blight was observed in the untreated variant with 40.8% (Figure 4).

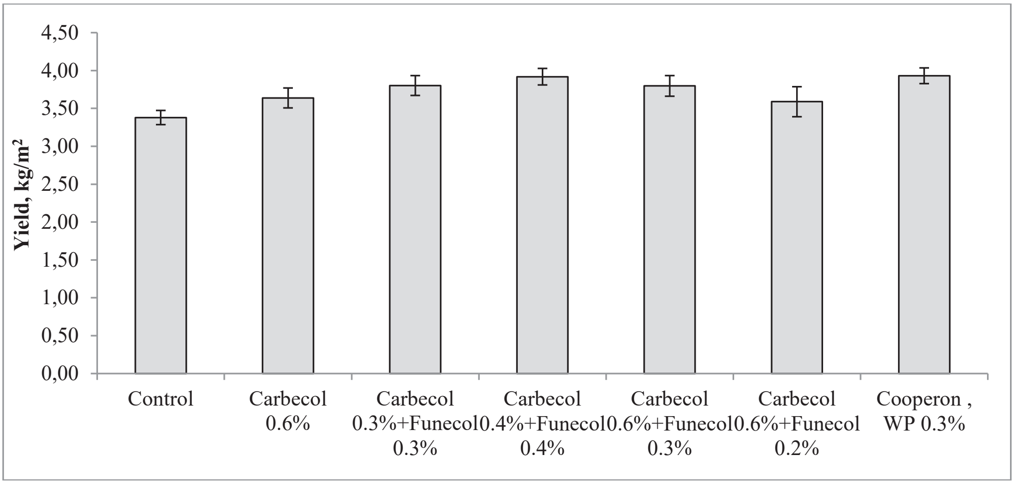

Figure 5. The effect of treatments with Carbecol alone or in combination with Funecol on the yield of tomato plants. Error bars represent SE at $p \leq 0.05$ of a mean pooled from three replications.

One of the objectives of the study was to determine the influence of treatments on fruits productivity of tomato plants. The application of different combinations of Carbecol and Funecol was also found to positively influence the fruit yield of tomato (Figure 5). For the untreated plants, control treatment recorded the lowest fruit weight compared to the other treatments. The effect of disease severity was negative on the yield of tomato fruits (Figure 5). Consequently, the mean yield in the untreated control was lower and different from the experimental treatments. Within the treatments, the total yield in Carbecol 0.4% + Funecol 0.4% treated plots was much higher and significantly different from other treatments at P = 0.05. For the plants with the integrated application of Carbecol and Funecol at a concentration of 0.4% for each biorational product, the highest fruit yield was recorded, resulting in a 16% increase in productivity compared to the control variant. It is necessary to note that Carbecol contains nitrogen and potassium nutrients which had a positive effect on growth and consequently the fruit yield of tomato plants increased. Similar observation was made by Abd-El-Kareem et al. (2012) who reported that the increase in yield of potato plants could also be due to application of products with bicarbonates during crop growth. Overall, the findings in this study could be useful for development of integrated disease management strategies of tomato cultivation. The protection products tested in this research offer new possibilities to enhance the capacity of tomato protection through the combined use of these biorational products. This approach could potentially reduce the rates and frequencies of chemical applications, consequently mitigating the negative impact on the environment (Šunjka & Mechora, 2022). Hence, the results of the current research revealed that integrated application of Carbecol and Funecol demonstrate comparable efficacy in controlling late blight of tomatoes. The treatment combining Carbecol 0.4% and Funecol 0.4% exhibited the lowest disease severity index and resulted in better tomato yields. However, it is essential to underline that their application does not provide complete protection against late blight. This study is the first to demonstrate the potential of biorational products Carbecol and Funecol in managing tomato late blight under greenhouse conditions. Generally, in reducing the severity of late blight disease and improving tomato fruit yield, the integrated application of Carbecol and Funecol was found to be more efficient than the application of Carbecol alone. We consider that further testing of these biopesticides is needed to investigate their efficacy in field conditions.

CONCLUSIONS

Study results suggest that spraying biofungicide Carbecol and the biofungicide Funecol has the potential to control late blight of tomato plants. The integrated use of these products decreased the severity of late blight in tomato plants by 50%. The most effective combination of Carbecol and Funecol to control late blight of tomato was Carbecol 0.4% + Funecol 0.4%. The combined application of Carbecol and Funecol, each at a concentration of 0.4%, enhanced the yield of tomato by 16%. Thus, the integrated application of plant protection ecological products should be considered an effective biotechnological option for eco-friendly management of late blight disease.

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THE INFLUENCE OF FOLIAR FERTILIZATION WITH HUMIC ACIDS - BASED PRODUCTS ON THE QUALITY OF TOMATO FRUITS

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Abstract

The use of natural biostimulators on crops is an innovative approach, friendly to the environment and with beneficial effects on the quality of production. In this sense, the objective of this study was to evaluate the application of the Lignohumate biostimulator to the tomato crop on fruit quality, depending on the fertilization dose. This is a bio-fertilizer, consisting of a complex of potassium salts of humic and fulvic acids, supplemented with microelements. The biological material was represented by the 'Giraffe F1' tomato hybrid. The specifics of the variants were: V1 (Mt) - unfertilized, V2 - fertilized with 1 g/10 L water, V3 - fertilized with 1.5 g/10 L water, V4 - fertilized with 2.0 g/10 L water. The monitored quality parameters were: total soluble substance, titratable acidity, reducing carbohydrates, ascorbic acid, total carotene, total lycopene and total polyphenols. The recorded data showed that all quality parameters had significant values compared to the control in the variants fertilized with Lignohumate 1.5 g/10 L water and 2.0 g/10 L water.

Key words: *humic substances, Lignohumate, Lycopersicon esculentum, quality.*

INTRODUCTION

A manner in which the use of fertilizers can be reduced without affecting the nutritional balance of plants is the use of biostimulants and natural extracts. Foliar application is widely used as a method to supplement the deficiency of nutrients that are difficult to decompose in the soil. This practice is used both for the application of macro and micronutrients and humic fertilizers, favouring the assimilation of nutrients by plants and increasing the yield and quality of crops (Iancu et al., 2019; Tejada et al., 2018).
Du Jardin (2015) stated that humic and fulvic acids and are part of the humic compounds in the soil, with the addition of humins and originating from soil organic matter decomposed by plants, animals and microorganisms. A series of fertilizers containing mainly humic or fulvic acids are considered organic fertilizers, which have an effect on plant growth and crop yield by improving nutrient absorption.
Humic substances, humic and fulvic acids are major components (65-70%) in organic matter from soil, determining plant growth, by increasing cell membrane permeability, intensifying respiration, photosynthesis, oxygen and phosphorus absorption, ensuring the growth of root cells (Shen et al., 2020; Abou & Husein, 2016). Abd El-Razek et al. (2020) stated that the application of humic acids directly to the soil improves soil structure and fertility, water retention capacity, increases microbial activity and cation exchange capacity.
Fulvic acid chelates and binds dozens of minerals into a bioavailable form used by the cells. These trace minerals serve as catalysts for vitamins inside the cell, fulvic acid being one of the most efficient transporters of vitamins into the cell (Abou & Husein, 2016).
Other authors have demonstrated that humic substances stimulate root growth and absorption of nutrients at vegetable crops (El-Nemr et al., 2012; Soare et al., 2018). In addition, these crops are less sensitive to stress conditions (drought, extreme temperatures, excessive moisture in the rhizosphere,

insufficient light and salinity) due to the high production of antioxidant compounds (Itelima et al., 2018). Unlu et al. (2011), through the foliar application of humic acids, to a cucumber crop, observed an increase in fruit and implicitly in crop yield. Soare et al. (2020) indicated that foliar application of Humusoil, a humic fertilizer product, had favourable effects on the genotypes of groundnuts cultivated on sandy soil from South of Romania. In addition, Matei et al. (2020) in their study showed that the level of the registered productions increased with the increase of the nutrient doses to grain sorghum comparative with unfertilized variants. According to other authors, the application of humic acids influenced the accumulation of carbohydrates and improved the colour and nutritional value of plum fruits (Lu et al., 2023), tomato (Dinu et al., 2013) and pepper (Apostol et al., 2022). In other studies, polyphenol extracts from seeds of *Vitis vinifera* were used along with humic acids, and the germination of tomato seeds was found (Dinu et al., 2014). Soare et al. (2017) found that humic acid applied to a cabbage crop significantly increased plant height, stem diameter, yield and quality.

Tomatoes (*Lycopersicon esculentum*) belong to the *Solanaceae* family and are among the most consumed vegetables in the world. The area cultivated with tomatoes, worldwide, was 4,917,735 ha in 2022, and the production reached 186.1 million tons. Currently, China is the largest tomato producing country in the world. In Romania, the area cultivated with tomatoes was 17,170 ha at the level of 2022, and the production was 298,920 tons (FAOSTAT, 2024). Tomato fruits contain high levels of antioxidants such as vitamin C, polyphenols, carotenoids and minerals, which are needed daily for a healthy diet. The interest in consuming high-quality fresh fruit is continuously increasing (Bădulescu et al., 2020). The levels of bioactive compounds are highly variable and can be influenced by variety, cropping system, harvesting stage and storage period (Dinu et al., 2017) and nutrient doses.

This study aimed to evaluate the application of the Lignohumate biostimulator organic to the tomato crop on fruit quality, depending on the fertilization dose.

MATERIALS AND METHODS

The researches were established in the experimental field of the Faculty of Horticulture in Craiova (Romania), in 2022, to evaluate the influence of the foliar application of the organic biostimulator Lignohumate on the quality of tomato fruits, depending on the fertilization dose. Lignohumate is a mixture of potassium salts of humic and fulvic acids, supplemented with trace elements. The product was applied in 3 stages, 10 days between treatments and in three different doses. The specifics of the variants were: V1 (Control) - unfertilized, V2 - fertilized with Lignohumate 1 g/10 L water, V3 - fertilized with Lignohumate 1.5 g/10 L water, V4 - fertilized with Lignohumate 2.0 g/10 L water.

The biological material was represented by the ‘Giraffe F1’ tomato hybrid. The culture was established by seedlings produced in a warm greenhouse, of 55 days. Planting was done in the last decade of April, at a distance of 0.9 m between rows and 0.3 m between plants. The crop, at planting, was fertilized with the organic fertilizer Orgevit 4-2.5-2.3 in a dose of 1.5 t/ha.

In order to carry out the quality analyses of the fruits, they were harvested by variants and repetitions and prepared according to the standards.

Analytical Methods

The total soluble substance (TSS) content was determined using an Optical Digital Handheld Refractometer Dr301-95 set at t = 20^0C.

The determination of titratable acidity (TA)

From a sample of 5-10 g of tomatoes homogenated with a vertical blender Braun MR 404 Plus for 1 minute, 1-2 mL were taken which were diluted in 10 mL of distilled water and titrated with 0.1 N sodium hydroxide in the presence of phenolphthalein.

The acidity calculation is made using the formula:

AT (%) = V x N x 100/m

V - volume of NaOH solution used for titration, (mL); m - sample weight (g); N -normality of NaOH solution.

Reducing sugars (%) were extracted in distilled water (1: 50 w/v) and assayed colorimetric with 3,5-dinitrosalicylic acid.

The determination of ascorbic acid

A sample of 10 g of tomatoes, previously ground with quartz sand has been transferred into a 100 mL balloon by using a solution of 2% hydrochloric acid. It has been stirred and after sedimenting it has been filtered into a dry glass. A 10 mL aliquot has been passed into a Berzelius glass, to which 30 mL of distilled water, 5 mL of 1% potassium iodate and 1 mL solution of starch have been added. It has been then titrated with potassium iodate N/250 and stirred until becoming bluish.

The calculation of ascorbic acid concentration is made by using the equation:

Ascorbic acid mg/100 g FW = 352 x V.f/m,

where: 352 = 3.52 x 100; 3.52 mg acid ascorbic which corresponds to 1 mL potassium iodate N/250 used for titration and 100 = reporting per 100 g FW; V - volume solution used for titration (mL); f - the factor of the potassium iodate N/250; m - sample weight (g).

The total phenolics content (TPC) was determined colorimetric at 765 nm by the Folin-Ciocalteu method. Gallic acid was used to plot standard curve and the results were expressed as mg of gallic acid equivalents (GAE)/ 100 g FW.

Lycopene and β-carotene were extracted in 2:1:1 hexane: methanol:acetone. The non-polar layer was collected and spectrophotometrically analyzed (Evolution 600 UV-Vis; Thermo Scientific, UK).

The contents of lycopene and β-carotene were calculated according to the following equations: lycopene mg/100 mL = $-0.0458 \times A663 + 0.204 \times A645 + 0.372 \times A505 - 0.0806 \times A453$;

β-carotene mg/100 mL = $0.216 \times A663 - 1.220 \times A645 - 0.304 \times A505 + 0.452 \times A453$.

The results were expressed in mg/100 g FW.

Statistical Analysis. The data obtained were analysed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), followed by the Dunnett's test and 95% Confidence.

RESULTS AND DISCUSSIONS

In tomatoes, taste is the main factor influencing consumer preferences. This largely depends on the content of total soluble substances, reducing carbohydrates and organic acids which are often used as important indicators for fruit flavour evaluation (Zhang et al., 2023). Fruits with a high dry matter concentration have better taste, higher processing yield, better transportability and keeping quality during storage. In this study, the total soluble substance (TSS) recorded variations from 3.3 to 5.2°Brix, and the total acidity from 0.347% to 0.454%, the highest values being recorded in the variant treated with Lignohumate with 2 g/10 L. The results in the present study are in accordance with the studies carried out by Sun et al. (2022) who found that as the application dose of humic acids increases in cherry tomato plants, the TSS content in the fruits also increases. Molla et al. (2012) in their study on the effects of applying biofertilizers on tomatoes showed an increase in sugar content (5.11 mg/100 g) through the combined use of household waste composted with *T. harzianum* T22. Some authors noted that applying humic acids to the soil at a dose of 1.5 g/L significantly improved the content of total soluble substances, total acidity and total sugar content in tomato fruits, 'Tessera' cultivar (Alenazi & Khandake, 2024).

Regarding the reducing sugar content, it varied from 2.58% (V1) to 3.14% (V4), and in this case it was found that in the variants treated with the Lignohumate biostimulator, a higher amount of reducing sugars was accumulated, but insignificant values were recorded between variants, from a statistical point of view ($P < 0.05$) (Table 1).

Table 1. The effect of applying the Lignohumate biostimulator on total soluble solids, titratable acidity and reducing sugar

Variant	TSS (°Brix)	Total acidity (%)	Reducing sugar (%)
V1 (Ct)- unfertilized	3.3 A	0.347 A	2.58A
V2- fertilized with 1 g/10 L of water	3.7 A	0.381 A	2.73A
V3- fertilized with 1.5 g/10 L of water	4.8*	0.429*	2.80A
V4- fertilized with 2 g/10 L water	5.2*	0.454*	3.14A

Means not labelled with the letter A are significantly different from the control level mean, P≤5.

In the present study, the foliar application of humic acids indicated positive effects on the ascorbic acid content, which varied from 16.20 (mg/100 g FW) (V1) to 22.40 mg/100 FW (V4). The highest values were recorded in the fruits harvested from the variants treated with Lignohumate, while the tomato fruits harvested from the control variant had the lowest values (Table 2). The total content of ascorbic acid increased significantly with the increase in the dose of Lignohumate (P≤5), the highest value being recorded at the dose of 2 g/10 L water. The results are in agreement with those reported by other authors as well. Alenazi and Khandake (2024), demonstrated that the foliar application of humic acids (HA) improves the content of vitamin C in the fruits harvested from the treated variants, higher values being recorded at the dose of 2 g/L of HA.

Table 2. The effect of applying the Lignogumate biostimulator on the biochemical composition

Variant	Ascorbic acid (mg/100 g FW)	Total lycopene (mg/100 g FW)	Total carotene (mg/100 g FW)	Total phenolics content (mg GAE/100 g FW)
V1 (Ct) - unfertilized	16.20A	6.40A	1.21A	24.53A
V2 - fertilized with 1 g/10 L of water	18.22*	6.82A	1.44A	26.81*
V3 - fertilized with 1.5 g/10 L of water	18.75*	7.24A	1.83*	29.42*
V4 - fertilized with 2 g/10 L water	22.40*	8.51*	1.76*	28.19*

Means not labelled with the letter A are significantly different from the control level mean, P≤5.

In previous studies carried out by the authors of this paper, in different melon hybrids, it was found that the higher the concentration of Lignohumate, the better the accumulation of nutrients (Dinu et al., 2019; Soare et al., 2018). According to He et al. (2022), the application of humic/fulvic acids to the lemon crop determined the increase in the content of vitamin C, total sugar and total soluble substance, in the treated variants, compared to the control variant ($p < 0.05$).

Regarding the total lycopene content, values were recorded between 6.40 and 8.51 mg/100g FW (at V1 and V4 respectively), finding that the application of the biostimulator based on humic and fulvic acids induces an increase in the total lycopene content in all variants compared to the control variant. These results suggest that the application of humic acids can contribute to enhancement of the efficiency in the absorption of major nutrients, especially based on K from the soil, and which improves the quality of tomato fruits.

The total carotenes in tomato fruits varied from 1.21 mg/100 g FW in the unfertilized variant to 1.83 mg/100 g FW in the variant fertilized with 1.5 g/10 L water (Table 2). It is also found that in the case of this component, variable accumulations are recorded, being influenced by the applied dose with Lignohumate based on potassium humate.

Regarding the content of total polyphenols, it varies from 24.53 mg GAE/100 g FW to 29.42 mg GAE/100 g FW. These values indicate a higher accumulation in all the treated variants compared to the control variant. This fertilizer induced positive effects on the quality of tomato fruits. He et al. (2022), reported a higher content of total polyphenols in the peel, pulp and seeds of lemons, in variants treated with humic acid fertilizers compared to the control variants, at three different harvest periods ($p < 0.05$).

CONCLUSIONS

The results of the research showed that the use of the natural biostimulator Lignohumate, based on potassium salts of huic and fulvic acids, applied in three different doses to the tomato crop induced positive effects on the quality of the tomato fruits. In particular, in the variants treated with Lignohumate, the content of total soluble substance, total acidity and reducing sugar increased, the values being significant compared to the control (Ct.) ($p < 0.05$), highlighting in particular the variant fertilized with a dose of 2 g/ 10 L of water. The accumulation of ascorbic acid, total carotene, total lycopene and total positive effects on the quality of the tomato fruits. Regarding the accumulation of ascorbic acid, total carotenes, total lycopene and total polyphenols was more evident both at variants with 1.5 g/10 L and at 2 g/10 L.

It can be stated that fertilization with Lignohumate is a viable alternative to organic culture for obtaining higher quality yields compared to the unfertilized, at cultures in the greenhouse where large amounts of chemical fertilizers are applied that can cause pollution of the entire trophic chain (soil, underground water, plants).

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A RESEARCH ON THE USE OF ORGANIC FERTILIZERS APPLIED TO TOMATO PRODUCTION

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Abstract

Studies have been conducted with regard to the way in which the elements of tomato field crop yield are influenced by the application of supplementary fertilization with organic fertilizers obtained only from natural ingredients. The organic material used was TAMARIS F1, an early tomato hybrid, recommended for greenhouse, solarium and field palisade cultivation. Based on the analysis of the experimental variants, it was found that the variant additionally fertilized with Plantella Bio had an average fruit weight ranging between 170-180 g, with a yield of 40 t/ha as compared to the control variant where the average fruit weight was between 130-140 g and the yield was 28 t/ha,. Consequently, there was a 30% higher yield in the variant fertilized with Plantella Bio than in the reference variant.

Key words: *Plantella, Agrecol, Tamaris, hybrid.*

INTRODUCTION

The research focused on the application of plant beneficial microorganisms to partially replace the use of chemical fertilizers is on the rise due to the requirement of sustainable agricultural development (Lin Ye et al., 2020) and studies have found that inoculation with selected native rhizobacteria has increased the quality of tomato seedlings; they can be used as bio-inoculant to achieve integrated nutrient management (María Micaela Pérez-Rodriguez et al., 2020). Application of organic fertilizers to the cherry tomato crop has increased the microbial biomass and improved nutrient movement, such as nitrogen movement activity and phosphorus movement activity (Takamitsu Kai et al., 2020). Application of compost-based fertilizer (derived from corn, cassava hulls and poultry manure) increased fruit yield by 145% compared to the control variants (Lateef Bamidele Taiwo et al., 2007). Organo-mineral fertilizers are a combined source of nutrients that can be effectively used to increase the long-term productivity of the tomato plant. (Adama Traore et al., 2022)

When compared to other vegetables such as cabbage and potatoes, tomatoes contain significant amounts of protein and carbohydrates and small amounts of fibre. In the US, tomatoes are a very important vegetable, whereas in Germany tomato consumption is no higher than cabbage consumption (Nillson T., 2005). The high value of tomatoes also lies in the fact that this vegetable is consumed very much in its raw state, so that the vitamins and mineral salts it contains are almost entirely used by the body. In terms of dietary value, the main points of interest are the sugar content (around 3-4%), vitamin C (20-60 mg per 100 g) and vitamin A (2-6 mg), and less so the energy value, which is quite low (176 calories) compared to other foods. We should not overlook the favourable proportion of amino acids and organic acids found in tomato fruit, as well as the magnesium, sodium, potassium and iron salts which are also found in an appropriate percentage for the proper functioning of the human body.

Nowadays, there is a great demand for environmentally friendly agriculture, for the production of quality and healthy food to feed the continuously growing population. (Pradeepa Jayasinghe, 2016) and in this context research has shown that the application of poultry manure has greatly increased the production and quality of fruit and chemical nitrogen fertilizers could be largely replaced with poultry manure (Yang Tao et al., 2022).

Research shows that the combined application of chemical fertilizers with organic fertilizers, specifically manure, stimulates plant growth, increases tomato yield and improves soil fertility in clay-textured soil. (Muhammad Hasnain et al., 2020). Studies have also shown that following organic fertilization, the quality of tomato fruit is superior, with increased lycopene content (Dimitrios Bilalis et al., 2018).

In the current context of environmentally friendly agriculture where it is recommended to reduce the use of chemical fertilizers and pesticides, we aimed, by this paper, at finding environmentally friendly alternatives to fertilize tomato crops. The hypothesis that we started from is to demonstrate that organic fertilizers depending on their chemical composition can influence differently the growth and fruiting of tomato plants and the novelty of the research is related to the use of an organic fertilizer Bio Plantella with seaweed extract as main component.

MATERIALS AND METHODS

The biological material used was the hybrid TAMARIS F1, produced by Clause Vegetable Seeds, an early tomato hybrid, recommended for greenhouse and solarium cultivation as well as for field, palisade cultivation. It has a high resistance to diseases and pests and a good tolerance to adverse conditions.

Three organic fertilizers have been used: Plantella Bio Organik produced by Unichem Slovenia, which is a long-lasting universal organic fertilizer made from poultry compost and comes in pellets. It improves soil fertility and increases soil yield and contains : N - 5%; P - 3%; K - 2%; CaO - 9%; MgO - 1% and microelements: B, Cu, Fe, Mn, Mo, Z. The second fertilizer was Bio Plantella produced by Unichem Slovenia, in liquid form, with the main component being seaweed extract (*Ascophyllum nodosum*), added vitamins, natural hormones and a mix of nutrients, amino acids, carbohydrates. The third fertilizer used was Agrecol organic vegetable fertilizer produced by the Polish company Agrecol Sp. z.o.o. high quality granular fertilizer with chemical composition nitrogen (N) - 6%, phosphorus (P2O5) - 5%, potassium (K2O) - 10% and magnesium (MgO) - 4%, ideal for soil preparation before sowing and planting.

The experiment had 4 variants, each variant with 3 repetitions, randomly assigned. In all variants, the basic fertilization was based on manure at a dose of 40 t/ha, the control variant was fertilized only with manure and in the other 3 variants Plantella Bio Organik, Bio Plantella and Agrecol legume were applied, these fertilizers were applied several times during the growing season, at an interval of 2 weeks between applications, making a total of 7 applications.

The research methods used were quantitative methods by means of which we were able to determine to what extent the use of different fertilizers based on natural ingredients influences the process of plant growth and the size of productivity elements.

For this purpose, measurements were made on the development of plant height by means of a tape measure every 10 days. The measurements also focused on the average number of fruits per plant by counting and on the average fruit weight by weighing.

RESULTS AND DISCUSSIONS

The tomato crop was planted on May 12, 2019, on a vermic, phreatic, moist, moderately carbonate chernozem soil overlaying loess, clay loam soil type in Ciocile commune, Chichinetu village, Braila county, using 67 days old seedlings, employing the following planting scheme: 75 cm between rows and 30 cm between plants per row. The purpose of the observations was to determine the influence of fertilization with products obtained from natural ingredients on the elements of productivity in the tomato crop grown in the field.

In terms of climate, during the growing season, rainfall was below the multiannual monthly average, except in June, when rainfall was above the multiannual monthly average. From a temperature point of view, during the entire growing season, the average monthly temperatures were above the multiannual monthly average.

As different growth rates of tomato plants were observed after fertilizer application, determinations were made on the evolution of plant height, the results of which are shown in Figure 1.

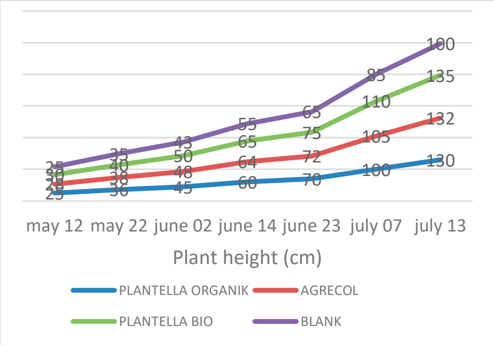

Figure 1. The evolution of plant height in the analyzed variants

From the graphical representation presented in Figure 1, it can be observed that among the organic fertilizers used compared to the variant fertilized only with manure, the most significant increases were recorded in the variant fertilized with Plantella Bio fertilizer. Thus, the plant height was 135 cm compared to only 100 cm for the control variety fertilized only with manure in the basic fertilization.

According to the graphical representation shown in Figure 2, the number of fruits per plant is also higher in the Plantella Bio fertilized variety than in the exclusively manure fertilized variety by 40%. The main component of this fertilizer is seaweed which strengthens the root system of the plants, ensures healthier plant growth and richer flowering.

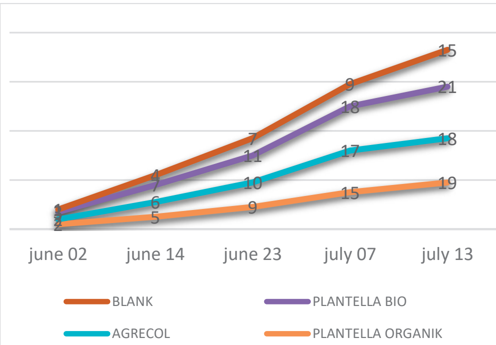

Figure 2. The evolution of the number of fruits per plant in the analyzed variants

Based on the reported results, it can be concluded that when compared to the control variant, which was fertilized only with manure, in the experimental variants analyzed there is a positive correlation between the plant height and the number of fruits per plant, thus, the variant fertilized with Plantella Bio delivered 21 fruits per plant, against only 15 fruits in the control variant, the variant fertilized with Plantella Bio being also the tallest plant, specifically 135 cm, which indicates that the stimulation of growth is also reflected in the stimulation of fruiting.

The productivity elements defined were the average number of fruits per plant, the average fruit weight and the average yield per plant.

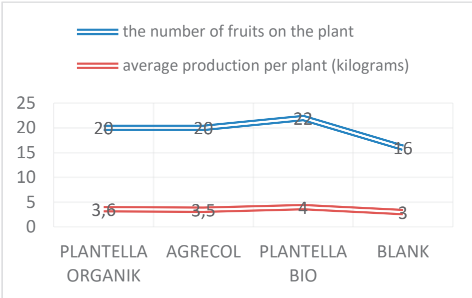

Figure 3. The correlation between the average number of fruits per plot and the weight of fruits per plant (kilograms)

Figure 3 shows that the yield per plant was 1 kg higher in the Plantella Bio fertilized variant than in the control variant, whereas, in the Plantella Organic fertilized variant, the difference compared to the control variant, was 0.6 kg/plant while in the Agrecol vegetable fertilized variant, the difference was 0.5 kg/plant.

As regards the determination of the average weight of tomato fruits and production, the results obtained show that the average weight of the fruits in the Plantella organic fertilized variety was 180 grams and the production was 40.33 tons per hectare compared to only 130 grams and 28.10 tons per hectare in the variety fertilized only with manure.

In order to see if the differences recorded between the variants analysed are statistically assured, we used the statistical analysis program JASP, the analysis of the variants was carried out using the ANOVA test, the results of which are shown in Table 1.

Given that $p<0.001$ is below the 0.05 significance threshold, the difference is highly significant (99.9% significance threshold), the coefficient of variation values are reported in Table 2.

Table 1. ANOVA - Production

Cases	Sum of Squares	df	Mean Square	F	p
Variant	249.389	3	83.130	1017.915	<0.001
Residuals	0.653	8	0.082		

Note. Type III Sum of Squares TyIIISum

Table 2. Descriptives - Production

Variant	N	Mean	SD	SE	Coefficient of Variation
AGRECOL	3	32.000	0.200	0.115	0.006
BLANK	3	28.100	0.200	0.115	0.007
PLANTELLA BIO	3	40.333	0.473	0.273	0.012
PLANTELLA ORG	3	36.067	0.153	0.088	0.004

Figure 4 shows the yields of each repetition (tons) in the experimental variants analysed.

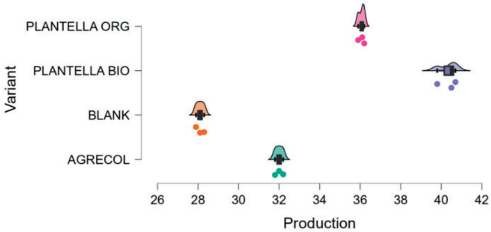

Figure 4 Yield per repetition of the variants analysed (tons/hectare)

In order to check the homogeneity of the dispersion, Levene's test was performed, and Figure 5 is a graphical representation of the homogeneity of the dispersion showing that there is no systematic deviation from the straight line.

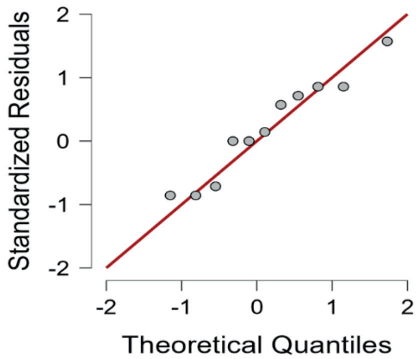

Figure 5 Homogeneity of dispersion

In order to determine the extent of the differences among the variants, Post Hoc tests have been carried out and the results are shown in Table 3.

Table 3 Post Hoc Comparisons - Variant

		Mean Difference	95% CI for Mean Difference Lower	Upper	SE	t	p_{tukey}	p_{bonf}
AGRECOL	BLANK	3.900	3.153	4.647	0.233	16.714	< .001	< .001
	PLANTELLA BIO	-8.333	-9.081	-7.586	0.233	-35.714	< .001	< .001
	PLANTELLA ORG	-4.067	-4.814	-3.319	0.233	-17.429	< .001	< .001
BLANK	PLANTELLA BIO	-12.233	-12.981	-11.486	0.233	-52.429	< .001	< .001
	PLANTELLA ORG	-7.967	-8.714	-7.219	0.233	-34.143	< .001	< .001
PLANTELLA BIO	PLANTELLA ORG	4.267	3.519	5.014	0.233	18.286	< .001	< .001

Note. P-value and confidence intervals adjusted for comparing a family of 4 estimates (confidence intervals corrected using the tukey method).

The results of the Post Hoc tests presented in Table 3 indicate that the greatest differences in yield are between the variant fertilized only with manure and the variant in which, in addition to the basic fertilization with manure on vegetation, the Plantella bio fertilizer was also applied.

CONCLUSIONS

As a result of the determinations carried out, it may be said that all the fertilizers used had positive effects on the tomato production obtained, but the Plantella organic fertilizer in liquid form was particularly remarkable, having as its main component seaweed extract which, according to recent studies, contains a lot of nutrients such as nitrogen, phosphorus, potassium, plant growth hormones and oligoelements.

Based on the results obtained, we recommend the use of fertilizers based on natural ingredients in the fertilization of tomato crops. These results are to be followed by an analysis of the influence of fertilizers based on natural ingredients on the quality of tomato fruit.

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BIOLOGICAL CONTROL OF MAIN DISEASES AND PESTS ON MELON CROPS IN THE FIELD

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Abstract

The main foliar diseases of melons, Pseudoperonospora cubensis and Sphaerotheca fuliginea, have a negative impact on fruit yield and quality. Also, in warm and dry summer, the mites (Tetranychus urticae) caused significant damages on melons crop in the field. This experiment aimed to study the efficacy of biological plant protection products for the control of mites, powdery and downy mildew on melons crops in field. The experiment includes 2 fungicides for downy mildew based on aluminum fosetyl, Mimosa tenuiflora extract and citrus, 2 biological fungicides for powdery mildew, based on Bacillus amyloliquefaciens Bacillus pumilus and 4 biological products used to mites control, based on potassium salts, mixture of terpenoids, cinnamon extract and saponified oil extract from Neem tree. The biological products had efficacy between 56.25 and 88.25% in the control of the adult mite T. urticae, 47.02-66.48% on P. cubensis and 71.25-84.76% on S. fuliginea. It is concluded that it is possible to grow melon in an organic way using these biological products for controlling the main diseases and pest on melons crop in the field.

Key words: *pathogen, mite, downy mildew, powdery mildew, Tetranychus urticae.*

INTRODUCTION

The cultivation of melons occupies an important area in Romania, with 4250 ha and 62900 tons production. In 2020, in Europe, our country ranks 4th in terms of area after Italy, Spain and France and 6th in terms of yield after Spain, Italy, France, Portugal and Greece (FAO, 2020).

The main foliar diseases of melons, *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev and *Sphaerotheca fuliginea* (Schlecht. Fr.) Pollacci, have a negative impact on fruit yield in Romania (Velichi, 2009; Sovarel et al., 2019), USA (Colucci and Holmes, 2010; Savory et al., 2011; Ojiambo et al., 2015), Europe and Asia. Also, in warm and dry summer, the mites (*Tetranychus urticae*) caused significant damages on melons crop in the field. The antagonists *Trichoderma viride*, *Gliocladium virens, Enterobacter cloacae*, saprofitic *Phytium olygandrum* can be used for seed treatment, soil introduction, pouring and sprays of plants for control of same important diseases on vegetable crops in protected facilities (Krasteva and Panayotov, 2009).

Serenade (*Bacillus subtilis* strain QST 713) are good efficacy (87%) on bacterial spot disease on greenhouse pepper (Sević et al., 2016).

Bacillus subtilis (B1) were effective against four major postharvest pathogens of muskmelon: *Alternaria alternata, Fusarium semitectum, Rhizopus stolonifer* and *Trichothecium roseum* (Yang et al., 2006).

Isolate T39 of *Trichoderma harzianum* is a biocontrol agent which controls the foliar pathogens, *Botrytis cinerea, Pseuperonospora cubensis, Sclerotinia sclerotiorum* and *Sphaerotheca fusca* (syn. *S. fuliginea*) in cucumber under commercial greenhouse conditions (Elad, 2000).

Trichoderma aggressivum f. *europaeum* TAET1 isolates inhibited *Botrytis cinerea, Sclerotinia sclerotiorum* and *Mycosphaerella melonis* growth by 100% in detached leaves assay and inhibited germination of *S. sclerotiorum* sclerotia (Sánchez-Montesinos et al., 2021).

Paecilomyces variotii is a potential biological control agent to be used against several aerial and soil diseases, thus it should be integrated into modern pest management strategies (Moreno-Gavira et al., 2021).

Powdery mildew incidence is reduced by soil surface applications with organic bio-fertilizer and organic materials such as wheat straw, and by foliar sprays with a fermented garlic preparation (Qin et al., 2011).

Tetranychus urticae Koch known as two-spotted spider mite is a polyphagous pest, has over than 200 host plant species (Kheradpir et al., 2007) and causes important economic loss all over the world (Abdallah, 2015). In Romania this pest attacks crops like eggplants, peppers, cucumbers, beans, tomatoes etc. (Călin et al., 2017). The mites are found on the underside of the leaf, where they feed by stinging and after the attack the leaves turn yellow and dry out (Camps et al., 2014).

Because it has a fast growth rate, a short life cycle and a high reproduction rate, it can very quickly reach a very high population density, leading to a qualitative depreciation of plants and a decrease in yields (Hanash et al., 2020).

There are many disadvantages to applying chemical acaricides, such as the development of resistance by the pest, damage to natural predators, phytotoxicity, environmental pollution and risks to human health (Gaber and Nasr, 2020). In order to reduce the populations of this pest and the need for pesticides, alternative control measures such as the application of biopesticides may be taken (Golec et al., 2020).

Biopesticides are control products with microbial, botanical, mineral or synthetic origin (Golec et al., 2020) with low mammalian toxicity, short persistence in the environment, safety for beneficial organisms, lack of harvest and re-entry restrictions and minimal risk for the development of resistance (Marcic et al., 2012).

MATERIALS AND METHODS

The experience was conducted in the field at the Research Development Institute for Vegetable and Flower Growing Vidra, in 2020. Planting was made on 2 June, using romanian melon (*Cucumis melo*) variety 'Festiv', sensitive to *Pseudoperonospora cubensis* and *Sphaerotheca fuliginea* pathogens. The experiment includes 2 fungicides for downy mildew based on aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}), check treated with chemical product), *Mimosa tenuiflora* extract 60% and citrus extract 20% (3 L ha^{-1}), 2 biological fungicides for powdery mildew, based on *Bacillus amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1}), *Bacillus pumilus* strain QST 2808 (10 L ha^{-1}) and 4 biological products used to mites control, based on potassium salts of C7-C20 fatty acids (16 Lha^{-1}), mixture of terpenoids QRD 460 (10 L ha^{-1}), cinnamon extract (3 L ha^{-1}) and saponified oil extract from Neem tree 40% (3 L ha^{-1}).

The experiment for pathogen control consists on 4 variants treated and check control.

1. Aluminum fosetyl 800 g kg^{-1}(2 kg ha^{-1}) + *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1})
2. Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) + *B. pumilus* strain QST 2808 (10 L ha^{-1})
3. *M. tenuiflora* extract 60% and citrus extract 20% (3 L ha^{-1}) + *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1})
4. *M. tenuiflora* extract 60% and citrus extract 20% (3 L ha^{-1}) + *B. pumilus* strain QST 2808 (10 L ha^{-1})
5. Check control.

Six foliar treatments were applied at 7 days intervals, first application was made before natural infection.

Observations have been made on the appearance and evolution of pathogens attack *S. fuliginea* and *P. cubensis*. The assessment parameters are diseases incidence (DI) - % infected leaves and severity of attack (SA) - % area infected of leaves. There were calculated: degree of attack (DA %) = (DI x SA)/ 100 and efficacy (E %) with Abbott formula (E= (1- DA treated/DA check) x 100. Sample /plot 100 leaves, natural field infection. Data were analysed using analysis of variance (ANOVA) and the means were separated by using Duncan's multiple range tests at P = 0.05.

To control the two-spotted spider mite to a melon crop in field were applied treatments every 7 days, with products (biopesticides) based on potassium salts of fatty acids C7-C18 479.8 g L^{-1} (47.98%), mixture of terpenoids QRD 460 which is based on natural extract from *Chenopodium ambrosioides* 152.3 g L^{-1} (15.23%), cinnamon extract 70% and saponified oil extract from *Neem* tree 40%. The EPPO standard PP1/037(2) was applied to count the pest. The number of eggs, nymphs and adults was recorded separately on three pre-marked plants assess at least 50 cm^2 leaf surface infested areas (by cutting 25 discs 1.5 cm in diameter). Analysis of variance was calculate using ANOVA and the effectiveness of the products using Abbott`s formula.

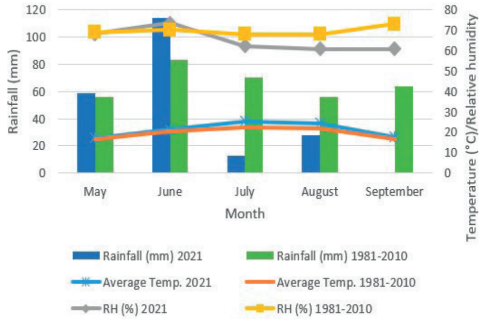

Figure 1. Average monthly temperature, relative humidity and rainfall between 1981 and 2010 and during 2021

Meteorological data during the growth season in 2021 and between 1981 and 2010 are presented in Figure 1. The average temperatures in 2021 were close to the multiannual average of 1981-2010. The rainfall deficit from July to September of 2021 (149 mm) determined a lower atmospheric humidity than the average from 1981-2010.

RESULTS AND DISCUSSIONS

The incidence of *P. cubensis* is lower on the variant treated with Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) than *M. tenuiflora* extract 60% + citrus extract 20% (3 L ha^{-1}). All leaves studied on check control were attacked by this pathogen, with 100% incidence and 38.35% severity. The diseases incidence on treated variants varied between 57.76% (Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) and 89.95% (*M. tenuiflora* extract 60% + citrus extract 20% (3 L ha^{-1}) (Table 1). The severity of attack on the treated variants varied between 19.97% (Aluminum fosetyl 800 g kg^{-1}) (2 kg ha^{-1}) and 22.58% (*M. tenuiflora* extract 60% + citrus extract 20%) (3 L ha^{-1}). Efficacy of product based on Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) is higher than *M. tenuiflora* extract 60% + citrus extract 20% (3 L ha^{-1}).

The incidence and severity of *S. fuliginea* has the lowest value (16.68%, respectively 16.17%) on variants treated with *B. pumilus* strain QST 2808 (10 L ha^{-1}). The incidence of disease is high (53,0%) and severity attack (34.92%) on check control. Efficacy of products is better on variants treated with *B. pumilus* strain QST 2808 (10 L ha^{-1}) than *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1}).

At 14 days after the first spray all the treatments proved significantly superior over control (Table 2). Neem oil was the best treatment in reducing the eggs (5.75 eggs/50 cm^2 leaf surface) and the adults (3.75 adults/50 cm^2 leaf surface) of mites population. The best treatment in reducing nymphs of the pest was cinnamon extract (9.75 nymphs/50 cm^2 leaf surface) followed by neem oil (9.75 nymphs/50 cm^2 leaf surface).

The results of this test showed a good efficacy of neem oil in reducing eggs (90.34%) and controlling the two-spotted spider mite adults (from 68.27 to 88.28%, Table 3), as obtained Hanash et al. (2020) and Cenușă et al. (2016).

Table 1. Efficacy of some biological products for *S. fuliginea* and *P. cubensis* pathogens

Variant	*Pseudoperonospora cubensis*				*Sphaerotheca fuliginea*			
	DI	SA	DA	E	DI	SA	DA	E
1	57.76^{b}	22.25^{b}	12.85^{b}	66.48^{a}	18.22^{b}	23.98^{ab}	4.37^{bc}	76.38^{b}
2	73.38^{ab}	19.97^{b}	14.65^{b}	61.80^{ab}	17.44^{b}	16.17^{b}	2.82^{c}	84.76^{a}
3	89.95^{a}	22.58^{b}	20.31^{b}	47.02^{b}	22.44^{b}	23.71^{ab}	5.32^{b}	71.25^{b}
4	85.12^{ab}	20.81^{b}	17.71^{b}	53.82^{ab}	16.68^{b}	17.10^{b}	2.85^{c}	84.59^{a}
5	100.00^{a}	38.35^{a}	38.35^{a}	-	53.00^{a}	34.92^{a}	18.50^{a}	-

DI = disease incidence (%); SA = severity of disease attack (%); DA = degree of attack (%); E = Efficacy (%).
Means followed by the same letter in the same column indicates the absence of significant differences at P=0.05 by Duncan's multiple range tests.

Table 2. Average number of eggs, nymphs and adults at 3, 7 and 14 days after treatments (%)

No	Treatments	Average number of eggs, nymphs and adults after treatments (%)								
		3 days			7 days			14 days		
		eggs	nymphs	adults	eggs	nymphs	adults	eggs	nymphs	adults
1.	Potassium salts of fatty acids	27.00^{b}	19.50^{c}	9.75^{b}	24.5^{b}	14.25^{b}	10.5^{b}	16.25^{bc}	10.0^{b}	12.25^{b}
2.	Natural extract from *C. ambrosioides*	22.00^{b}	10.00^{b}	7.75^{b}	23.5^{b}	11.00^{bc}	9.25^{b}	28.00^{b}	12.5^{b}	14.00^{b}
3.	Cinnamon extract	11.00^{c}	9.75^{b}	7.50^{b}	12.25^{b}	6.75d	5.75^{bc}	14.00^{bc}	6.25^{b}	5.75^{c}
4.	Neem oil	21.00^{b}	8.50^{b}	8.25^{b}	17.5^{b}	9.00^{cd}	3.50^{c}	5.75^{c}	9.75^{b}	3.75^{c}
5.	Control	48.50^{a}	39.00^{a}	26.00^{a}	51.25^{a}	40.50^{a}	27.5^{a}	59.5^{a}	41.25^{a}	32.00^{a}

Means followed by the same letter within each column are not statistically different, LSD ($p<0.05$) according to Duncan`s multiple range test (DMRT).

Table 3. Effectiveness of biopesticides at 3, 7 and 14 days after treatments (%)

No	Treatments	Effectiveness of biopesticides after treatments (%)								
		3 days			7 days			14 days		
		eggs	nymphs	adults	eggs	nymphs	adults	eggs	nymphs	adults
1.	Potassium salts of fatty acids	44.33^{c}	50.00^{b}	65.38^{a}	52.20^{b}	64.81^{c}	61.82^{b}	72.69^{ab}	75.76^{ab}	61.72^{b}
2.	Natural extract from *Chenopodium ambrosioides*	54.64^{b}	74.36^{a}	70.19^{a}	54.15^{b}	72.84^{bc}	66.36^{b}	52.94^{b}	69.70^{b}	56.25^{b}
3.	Cinnamon extract	75.77^{a}	75.00^{a}	71.15^{a}	76.10^{a}	83.33^{a}	79.09^{c}	76.47^{ab}	84.85^{a}	82.03^{a}
4.	Neem oil	56.70^{b}	78.21^{a}	68.27^{a}	65.85^{ab}	77.78^{ab}	87.27^{a}	90.34^{a}	76.36^{ab}	88.28^{a}
5.	Control	-	-	-	-	-	-	-	-	-

Means followed by the same letter within each column are not statistically different, LSD ($p<0.05$) according to Duncan`s multiple range test (DMRT).

Table 4. Yield difference between treatments with different active ingredient for controlling *P. cubensis* and *S. fuligiena* on melons

No.	Active ingredient	Yield (t ha^{-1})
1.	Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) + *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1})	28.32^{c}
2.	Aluminum fosetyl 800 g kg^{-1} (2 kg ha^{-1}) + *B. pumilus* strain QST 2808 (10 L ha^{-1})	28.86^{c}
3.	*M. tenuiflora* extract 60% and citrus extract 20% (3 L ha^{-1}) + *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1})	26.31^{b}
4.	*M. tenuiflora* extract 60% and citrus extract 20% (3 L ha^{-1}) + *B. pumilus* strain QST 2808 (10 L ha^{-1})	25.45^{b}
5.	Check control	21.36^{a}

Means followed by the same letter within each column are not statistically different, LSD ($p<0.05$) according to Duncan`s multiple range test (DMRT).

Table 5. Yield obtained (t ha^{-1}) at variants with products for *T. urticae*

No.	Active ingredient	Yield (t ha^{-1})
1.	Potassium salts of fatty acids	24.99^{bc}
2.	Natural extract from *Chenopodium ambrosioides*	23.97^{b}
3.	Cinnamon extract	26.10^{c}
4.	Neem oil	29.03^{d}
5.	Control	20.64^{a}

Means followed by the same letter within each column are not statistically different, LSD ($p<0.05$) according to Duncan`s multiple range test (DMRT).

Cinnamon extract had a efficacy of 82.03%, followed by variant with pottasium salts of fatty acids (61.72%). The lowest effectiveness in controlling the mite adults was registered at the variant with natural extract from *Chenopodium ambrosioides* (56.25%). The best efficacy in reducing nymphs was registered at variant with cinnamon extract (84.85%) followed by neem oil (76.36%).

The yield obtained had the highest values at variants with aluminum fosetyl + *B. pumilus* strain QST 2808 (28.86 ha^{-1}), followed by the Aluminum fosetyl (28.32 ha^{-1}) + *B. amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1}) compared to check control (21.36 t ha^{-1}) (Table 4).

Regarding the yield obtained, at all 4 variants good values of yields were registered in comparison with the check control (Table 5). The biggest value of yield was at variant with neem oil (29.03 t ha^{-1}) compared to check control (20.64 t ha^{-1}).

CONCLUSIONS

The biological products based on *Mimosa tenuiflora* extract 60% + citrus extract 20% (3 L ha^{-1}), *Bacillus amyloliquefaciens* strain FZB24 (0.37 kg ha^{-1}) and *Bacillus pumilus* strain QST 2808 (10 L ha^{-1}) may be used with good results for control of *Pseudoperonospora cubensis* and *Sphaerotheca fuliginea* on melon crops in the field.

Neem oil had the best efficacy in controlling eggs (90.34%) and adults (88.28%) of *T. urticae* and variant with cinnamon extract registered a good efficacy (84.85%) in reducing nymphs of the pest. The variant with natural extract of *Chenopodium ambrosioides* had a low efficacy for all stages of the pest. Also, the highest yield was recorded on the variant with neem oil (29.03 t ha^{-1}) compared to check control (20.64 t ha^{-1}).

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RELATIONSHIPS BETWEEN RGB COLOR CHARACTERISTICS AND TOMATO FRUIT QUALITY

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Abstract

The present study aimed to establish the relationship between the characteristics of RGB images of tomato fruits of three cultivars and important indicators of the quality of these fruits. In the study, 30 fruits from three cultivars at different ripening stages were used. The digital images of each tomato from four sides were obtained using a document camera. The same fruits were analyzed for dry matter, total dyes, vitamin C, titr. organic acids, beta carotene and lycopene content. The data of each image's G, R, and B channels were extracted and averaged. The average data of grayscale were taken as well. Based on the color characteristics and the chemical analyses, a procedure of descriptive statistics, correlation, and regression analysis was performed. The image procedure of all obtained data was performed using Jupyter notebook. The highest correlation coefficient (-0.860) was obtained between the Gray channel and total dye content, followed by the strong correlation between the Green channel and lycopene content (-0.820). And the models with the highest predictive ability were the models for total dye by Gray channel values (R^2 = 0.815) and lycopene by Green channel (R^2 = 0.761).

Key words: *tomato, images, color, Jupyter notebook, regression.*

INTRODUCTION

The tomato (*Solanum lycopersicum*) one of the economically important agricultural crops that are eaten fresh or processed. Colour or pigment changes during tomato ripening are characterized by loss of chlorophyll and rapid accumulation of carotenoids, especially lycopene. Colour is an important quality indicator of fruits and vegetables. Given the consumption of fresh fruit and the short shelf-life, there is a growing need for new, alternative technologies to assess the quality of such fruit and the possibility of using it in processing for fruit juices, jams, etc. Alternative technologies with the potential to sort fruit by appearance, texture, taste, nutritional value would provide higher quality fruit, increase consumer confidence and satisfaction, and increase the competitiveness and profitability of the agricultural and fruit industry (ElMasry et al., 2006., Hasanzadeh et al., 2022). In recent years, successful studies have been conducted to determine quality parameters of tomatoes at different maturity levels using portable spectrometers in the visible and near infrared range (Huang et al., 2018; Lu et al., 2016; Skolik et al., 2019).

The aim of the present study was to establish the relationship between the characteristics of RGB images of tomato fruits of three cultivars and important indicators of the quality of these fruits with the possibility to develop regression models for prediction.

MATERIALS AND METHODS

Tomato samples

In the paper is presented analysis of tomato fruits from three famous cultivars: Manusa, Mirsini and Red Bounty, grown in the greenhouse production base in Plovdiv,

Bulgaria. From each variety, 10 fruits of different maturity levels were taken. A document-camera is used for image data acquisition. A database of 120 digital images of the tomato fruits (40 from each variety) is formed. The digital images are acquired in the main color space RGB.
The dry matter, %, titratable organic acids, % calculated as citric acid after titration with 0.1 N NaOH, total pigments, mg %, vitamin C mg % by Tilman's reaction with 2,6-ichlorophenolindophenol, total dyes, mg %, lycopene, mg % (Manuelyan, 1991), and beta carotene, mg % content were measured on each fruit.

Color components of tomato friuts images

The software Jupyter notebook was used to process the images. Jupyter Notebook - browser based front-end originally developed for the programming languages Julia, Python. (Palkovits, 2020) Each image was cropped and the average values of R channel, G channel, B channel are taken through the corresponding code:

```
avgR = np.mean(mirsini1A_crop[:,:,0])
avgG = np.mean(mirsini1A_crop[:,:,1])
avgB = np.mean(mirsini1A_crop[:,:,2])

r,g,b = mirsini1A_crop [:,:,0], mirsini1A_crop[:,:,1],
mirsini1A_crop[:,:,2]
```

Each cropped image was represented in grayscale format by multiplying the values of the three channels by the corresponding coefficients via code:

```
mirsini1A_crop_gray = 0.299 * r + 0.587 * g + 0.144 * b
mirsini1A_crop_gray.mean()
```

Tomato fruits images from Mirsini cultivar (a), Manusa cultivar (b) and Red Bounty cultivar (c) are presented in Figure 1 after image processing.

Statistical analysis

Correlation analysis was used to examine the strength of the relationships between the examined chemical components of tomato fruits from different varieties (Manusa, Mirsini, Red Bounty) and the color components from their images.

Regression analysis was applied to calculate the predictive models defining relations between color components and chemical traits of tomato fruits. Two regression models were compared - Linear (L), (1) and Quadratic (Q), (2), expressed with the equations:

$$Y = b_0 x + b_1 \quad (1)$$
$$Y = b_0 + b_1 x + b_2 x^2 \quad (2)$$

where: Y are the observed parameters (Dry matter, Vitamin C, Titr. organic acids, Total dyes, Lycopene, and Beta carotene); x is the fixed factor (color components extracted from the tomato images); b_0; b_1; b_2 are the model coefficients. The models' estimation was done by comparing the parameters Coefficient of determination (R^2), Standard error of the estimation (SEE), and Standard error of predicted values (SEPv). The analysis and processing of the experimental data was done with the IBM® SPSS® Statistics 26.0 software at the significance level α=0.05

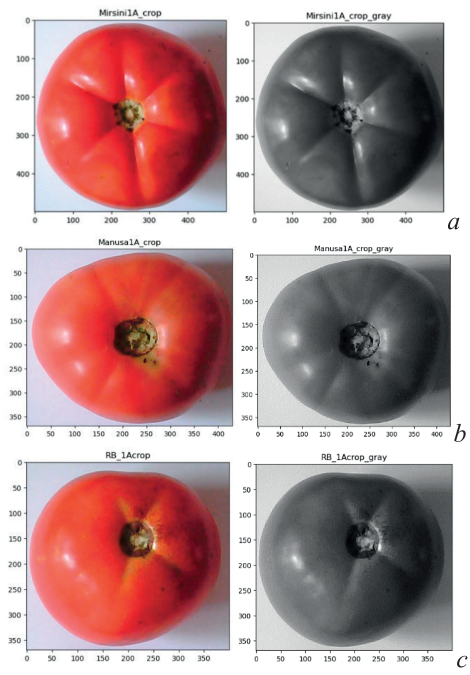

Figure 1. Tomato fruits from Mirsini var (a), Manusa var. (b) and Red Bounty var. (c)

RESULTS AND DISCUSSIONS

Correlation analysis

Descriptive statistics of average values of R, G, B, Gray channels and the studied chemical parameters of all tomato fruits are presented in Table 1.

Table 1. Descriptive statistics of color characteristics and the studied chemical parameters in all tomato fruits

	min.	max.	mean	SD
R mean	109.70	226.05	183.67	13.96
G mean	83.36	145.38	107.52	14.44
B mean	83.61	197.51	100.97	14.99
Gray mean	113.82	172.16	132.93	10.39
Dry matter, %	3.50	4.70	3.93	0.28
Vitamin C, mg %	13.10	31.00	20.38	4.01
Titr. organic acids, %	0.23	0.45	0.32	0.05
Total dyes, mg %	1.25	4.43	2.58	0.89
Lycopene, mg %	0.36	3.90	2.09	0.96
Beta carotene, mg %	0.00	2.43	0.31	0.42

Table 2 presents the results of the bivariate correlation analysis regarding the strength of the relationship between color components extracted from the tomato images and the investigated chemical components.

As can be seen from the table, in all three tomato cultivars studied, the correlation between the G (green) and Grey channel of the images with the chemical components studied was the strongest.

The highest correlation coefficient (-0.860) was obtained between the Gray channel and total dye, mg % content for the Manusa cultivar, followed by the strong correlation between the G channel and lycopene content, mg % in the Mirsini cultivar (-0.820) and between the Gray colour channel and lycopene content in the Manusa cultivar (-0.798).

Table 2. Bivariate correlation between colour characteristics and examined components of tomato fruits

	Dry matter (%)	Vit C (mg %)	Titr. organic acids (%)	Total dyes (mg %)	Lycopene (mg %)	Beta carotene (mg %)
			Manusa			
R	0,435	-0,008	-0,216	-0,599**	-0,634**	0,500*
G	0,096	-0,236	-0,176	**-0,774 ****	**-0,732 ****	0,179
B	0,309	-0,070	-0,302	-0,751**	-0,668**	0,318
Gray	0,210	-0,127	-0,282	**-0,860****	**-0,798****	0,314
			Mirsini			
R	-0,254	-0,305	-0,125	0,109	0,234	-0,365
G	-0,031	0,450*	0,398	**-0,774****	**-0,820****	0,664**
B	0,013	-0,015	0,323	-0,421	-0,435	0,270
Gray	-0,073	0,345	0,324	-0,645**	-0,753**	0,605**
			Red Bounty			
R	0,050	-0,503*	-0,017	0,218	0,246	-0,361
G	0,517*	0,684**	-0,456*	**-0,692****	**-0,691****	0,450*
B	0,485*	0,402	-0,496*	-0,397	-0,394	0,237
Gray	0,542*	0,532*	-0,476*	-0,620**	-0,611**	0,338

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

In the our study, a moderate correlation was also found between the G channel and total dye content for the three cultivars (-0.774; -0.774 and -0.692), and between the G channel values and lycopene content in cultivar Red Bounty (-0.691). Ropelewska et al. (2022) reported similar results, with a much higher degree of correlation between the green channel and lycopene content. The authors indicated a strong negative correlation, with values of r = -0.99. The authors also constructed calibration models with a high degree of accuracy for the determination of lycopene content based on the colour characteristics obtained from the G channel. What is interesting in their study is that they used tomato varieties of different colour and shape (yellow, orange, pink, red, including cherry tomatoes). These results are a prerequisite for creating predictive regression models for the investigated chemical components of the three tomato cultivars based on the green and gray components of their colour images.

Regression analysis

Linear and nonlinear regression equations were constructed based on data from laboratory analyses of the chemical components examined in the three tomato cultivars and the green and gray components of their colour images.

Dry matter (%), Vitamin C (mg %) and Titr. organic acids (%)

Table 3 presents the regression models estimated to predict the dry matter content of the studied tomato cultivars. For the cultivars Manusa and Mirsini, both linear and non-linear models were statistically insignificant and not suitable for predicting dry matter content. Only the models developed for the cultivar Red Bounty were statistically significant, with the highest coefficient of determination (R^2 = 0.323) being the quadratic model constructed for the green colour component. The coefficient of determination (R^2) indicated that 32.3% of the variation in dry matter in the Red Bounty cultivar was due to variation in the G values. Furthermore, SEE = 0.152 and SEP = 0.040 of this model are the lowest, which is a confirmation that it could be be used to predict the dry matter content in Red Bounty cultivar.

Table 3. Models summary and parameters estimation showing the relations between Dry matter (%) and Green and Gray channel values for the investigated tomato fruits

Parameter estimates		Equations	R^2	SEE	SEPV	Sig.
		Manusa				
Green	L	$DM = 0,001\,x + 3,688$	0,009	0,188	0,057	0,686
	Q	$DM = 1,803 + 0,038x - 0,0001x^2$	0,036	0,173	0,050	0,732
Mean gray	L	$DM = 0,004\,x + 3,311$	0,044	0,186	0,066	0,374
	Q	$DM = -5,447 + 0,139x - 0,0005x^2$	0,122	0,175	0,062	0,331
		Mirsini				
Green	L	$DM = -0,0007\,x + 4,204$	0,001	0,333	0,103	0,897
	Q	$DM = 1,626 + 0,048x - 0,0002x^2$	0,021	0,336	0,110	0,836
Mean gray	L	$DM = -0,003\,x + 4,474$	0,005	0,333	0,101	0,760
	Q	$DM = -8,950 + 0,205x - 0,0008x^2$	0,058	0,301	0,098	0,603
		Red Bounty				
Green	L	$DM = 0,007\,x + 3,067$	0,268	0,162	0,049	0,019
	Q	$DM = 0,237 + 0,058x - 0,0002x^2$	**0,323**	**0,152**	**0,040**	**0,036**
Mean gray	L	$DM = 0,012\,x + 2,275$	0,293	0,159	0,048	0,014
	Q	$DM = -4,174 + 0,108x - 0,0003x^2$	0,313	0,160	0,042	0,041

* Level of significance $p < 0.05$; R^2 - Coefficient of determination; SEE - Standard Error of the Estimate; SEPV - Standard Error of Predicted Value

Table 4 presents the regression models estimated to predict the vitamin C, mg % content of the studied tomato varieties. For the Manusa the regression models were statistically insignificant and not suitable for predicting vitamin C content. For the Mirsini only the models based on the G values were statistically significant. In these models, the quadratic and linear models have very similar coefficient of determination values (0.204 and 0.202), i.e. about 20% of the variation in vitamin C content in Mirsini var. is due to variation in the green colour channel. However, the quadratic model has lower values of both the SEE = 3.768 and the SEP = 1.385, making it more suitable than the linear model for predicting the amount of vitamin C in Mirsini var. For the cultivar Red Bounty, all fitted models were statistically significant. Again, the quadratic model based on the G colour component had the highest coefficient of determination (R^2 = 0.481), i.e. 48.1% of the variation in vitamin C in Red Bounty could be explained by variation in G values. Also, SEE = 1.287 and SEP = = 0.402 of this model are the lowest, i.e. it can be used to predict the vitamin C content in Red Bounty.

Table 4. Models summary and parameters estimation showing the relations between Vitamin C (mg %) and Green or Gray channel values for the investigated tomato varieties

Parameter estimates		Equations	R^2	SEE	SEPv	Sig.
		Manusa				
Green	L	$Vit\ C = 0{,}077\ x + 28{,}108$	0,056	4,396	1,344	0,317
	Q	$Vit\ C = 38{,}616 - 0{,}282\ x + 0{,}0008\ x^2$	0,057	4,376	1,365	0,607
Mean gray	L	$Vit\ C = -0{,}056\ x + 27{,}352$	0,016	4,487	1,365	0,594
	Q	$Vit\ C = -132{,}679 + 2{,}415\ x - 0{,}009\ x^2$	0,061	4,542	1,322	0,584
		Mirsini				
Green	L	$Vit\ C = 0{,}155\ x + 5{,}199$	0,202	4,573	1,406	0,047
	Q	$Vit\ C = 15{,}793 - 0{,}045\ x - 0{,}0009x^2$	**0,204**	**3,768**	**1,385**	0,044
Mean gray	L	$Vit\ C = 0{,}191\ x - 3{,}390$	0,119	4,806	1,459	0,136
	Q	$Vit\ C = 87{,}360 - 1{,}215x - 0{,}005x^2$	0,129	4,274	1,472	0,309
		Red Bounty				
Green	L	$Vit\ C = 0{,}091\ x + 9{,}829$	0,468	1,342	0,407	0,001
	Q	$Vit\ C = -3{,}35 + 0{,}326x - 0{,}001x^2$	**0,481**	**1,287**	**0,402**	0,004
Mean gray	L	$Vit\ C = 0{,}113\ x + 4{,}753$	0,283	1,558	0, 474	0,016
	Q	$Vit\ C = 87{,}987 - 1{,}125x - 0{,}005x^2$	0,318	1,543	0,479	0,039

* Level of significance $p < 0.05$; R^2 - Coefficient of determination; SEE - Standard Error of the Estimate; SEPV - Standard Error of Predicted Value

Similar to the models developed to predict dry matter content, only the models developed to predict titratable organic acid content of Red Bounty were statistically significant. The model with the highest coefficient of determination (R^2 = 0.405) was the quadratic model based on Green colour channel. The coefficient of determination indicates that 40.5% of the variation in titratable organic acid content in the cultivar Red Bounty depends on variation in the green colour component. Both the SEE (0.019) and SEP (0.003) of this model are very low, i.e., the model is suitable for predicting the content of titratable organic acids in Red Bounty cultivar.

Lycopene (mg %), Total dyes (mg %), and Beta carotene (mg %)

Figure 2 visualizes the estimated regression models for predicting Lycopene content, mg% in the studied tomato cultivars. Similar to the models for predicting Total dyes content, the estimated models for all three cultivars were statistically significant, with higher coefficients of determination and lower errors obtained for all quadratic models compared to the linear models. The quadratic models for tomato Manusa var. developed with the Gray channel data were characterized by a high coefficient of determination (0.761), i.e. 76.1% of the variation in Lycopene content in tomatoes of this cultivar could be explained by variation in the Gray values. The errors of the model created with the Gray component data (SEE = 0.300; SEPv = 0.087) were lower than those of the model estimated with the G channel.

For the other two cultivars Mirsini and Red Bounty, the quadratic models estimated on the basis of the Green colour component were more suitable for predicting the Lycopene content. Their coefficients of determination (0.675 and 0.485) are higher than the coefficients of the models for predicting Lycopene content obtained with the gray component. The errors of the prediction models for lycopene content in Mirsini tomatoes were SEE = 0.561 and SEPv = 0.173, and in Red Bounty tomatoes SEE = 0.580 and SEPv = 0.173, being lower in comparison with the errors of the quadratic models obtained with the gray colour component data. There are various successful studies related to the color used to measure lycopene (Villaseñor-Aguilar et al., 2021), in this respect the results obtained in our study were expected.

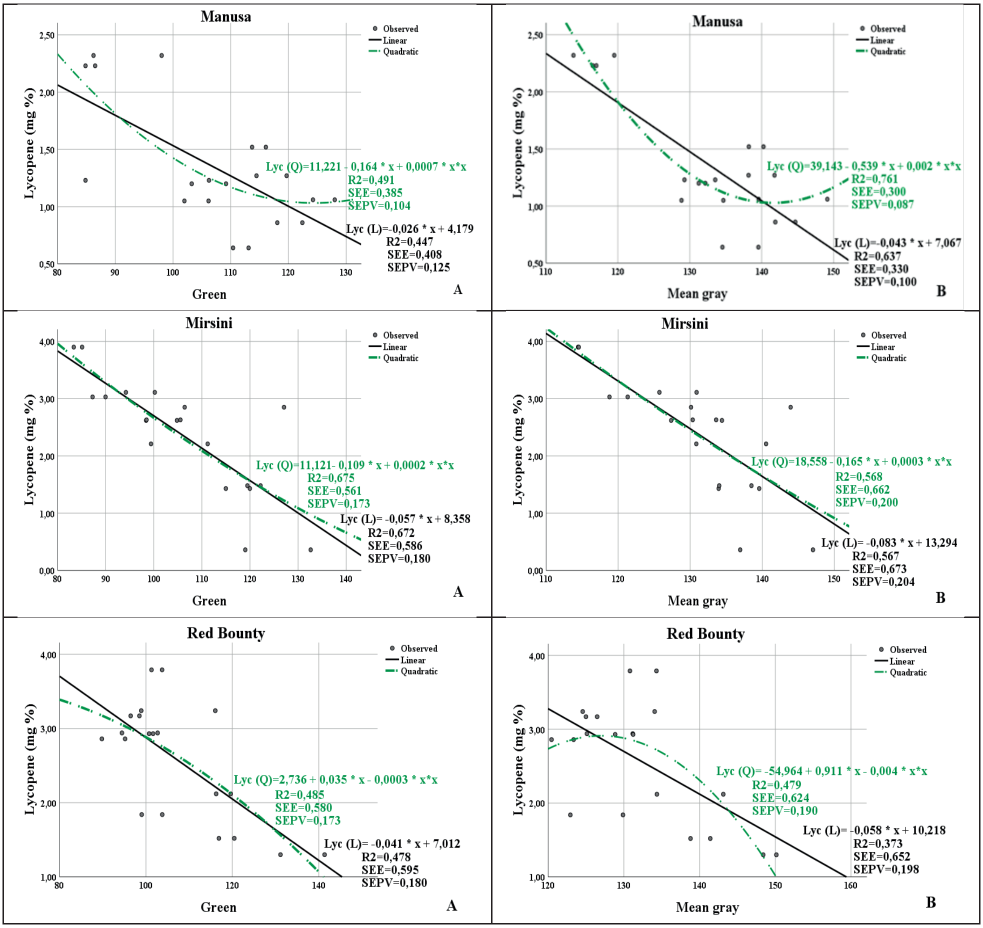

Figure 2. Estimation of the regression models' curves showing the relation between the content of Lycopene (mg %) and Green or Gray colour components for the investigated tomato varieties

Figure 3 visualizes the estimated regression models to predict the total dyes, mg% content in the studied tomato fruits. The resulting regression models for all three cultivars are statistically significant, but the quadratic equations have higher coefficients of determination and lower errors compared to the linear models. The quadratic regression models for tomato cultivar Manusa, developed with the data on the Gray component, had a very high coefficient of determination (0.815), i.e. 81.5% of the variation in total dyes, mg % content in this cultivar could be explained by variation in the Gray colour component. Accordingly, the errors of the model generated with the gray component data (SEE = 0.225; SEPv = 0.043) were lower than those of the model estimated with the green colour component.

For the Mirsini cultivar, the quadratic models estimated with the G colour values have higher values of both the coefficient of determination (0.611) and the corresponding errors (SEE = 0.524; SEPv = 0.157) when compared to the quadratic models obtained with the gray component data. The predictive models obtained for the total dyes, mg% content of Red Bounty had the same coefficient of determination values (0.488), but the quadratic model obtained with the green colour component data had lower errors (SEE = 0.547; SEPv = 0.170) compared to that obtained with the gray colour component, i.e. it was more suitable for predicting courts.

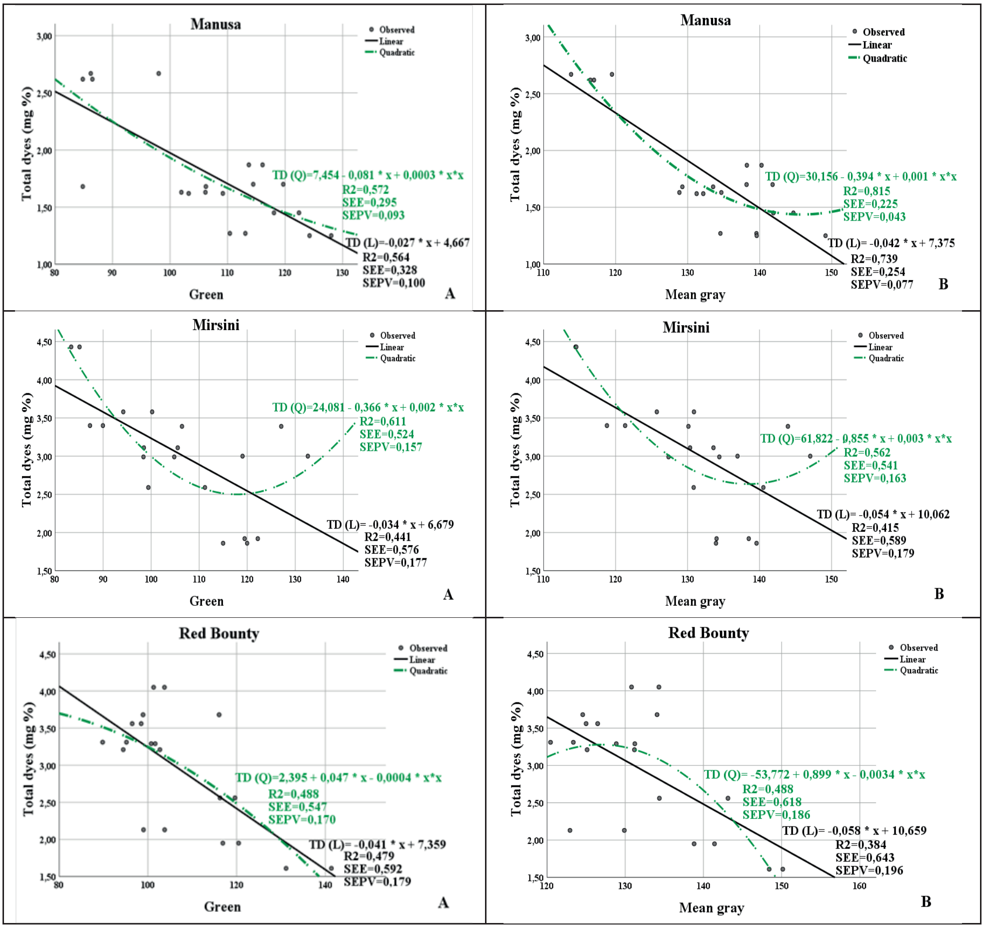

Figure 3. Estimation of the regression models' curves showing the relation between the content of Total dyes (mg %) and Green or Mean gray color components for the investigated tomato varieties

According to the Beta carotene, mg % prediction the results of the regression analysis show that the estimated models for the Manusa and Red Bounty cultivars were statistically insignificant.

All the models developed for the cultivar Mirsini were statistically significant, but as with the other chemical components studied, the one with the highest coefficient of determination (R^2 = 0.617) was the quadratic model constructed from the green colour component data.

The errors of this model are the lowest (SEE = 0.040 and SEPV = 0.012, respectively), i.e. the model is suitable for predicting the Beta carotene, mg % content in the Mirsini cultivar.

CONCLUSIONS

An experiment was conducted with tomato fruits of three different cultivars (Manusa, Mirsini and Red Bounty) to evaluate the relationship between their major chemical elements and colour components obtained from their images.

Using bivariate correlation analysis, it was found that the strongest correlation was between the Green and Gray colour components of the images with the chemical elements studied.

Regression models were created to predict the investigated chemical elements for the three tomato cultivars from the Green and Gray colour component data of their images.

It was found that the models with the highest predictive ability were the models for total dye, mg % and lycopene, mg % contents for all three tomato varieties.

This study complements previously known methods for non-destructive evaluation of major components of tomato fruit composition and highlights the potential of regression models to be integrated into automated tomato fruit harvesting, sorting, processing systems.

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SMALL HOLDINGS IN MOUNTAIN AND SEMI-MOUNTAIN AREAS IN BULGARIA AND THEIR IMPACT ON AGROBIODIVERSITY

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Abstract

Agrifood system includes the totality of the stakeholders, interactions and decisions that contribute to agricultural production. Small farms are highly vulnerable to climate change because most depend on weather conditions, especially rainfall, cultivate small areas in mountainous areas and hard-to-reach areas, and do not have access to technical or financial support. Local and traditional varieties play an important role, as a resource for breeding, sustainable crop diversity in farms or to compliment diets with organic products. Plant diversity grown in small holdings in Bulgaria is characterized by a large number of crops: vegetables, grain legumes, medicinal and aromatic species. The sustainable production of horticultural crops provides a key part of the healthy food. Our aim is to explore developing of conserved collections through new information strategies, supporting free access to plant gene fund. The study provides information on crop diversity in rural areas using the survey method and analysing the passport data from conducted collection missions. Based on results argent activity plan to improve protection of these valuable plant genetic resources for guarantee our food and agriculture will be recommended.

Key words: *climate changes, horticultural crops, landraces, data base, genebank.*

INTRODUCTION

Agri-food system includes the totality of the stakeholders, interactions and decisions that contribute to agricultural production. Small farms are highly vulnerable to climate change because most depend on weather conditions, especially rainfall, cultivate small areas in mountain areas and hard-to-reach areas, and do not have access to technical or financial support. This fact makes them priority areas for conservation (Matthies et al., 2023).

Plant genetic resources are cultivated and wild forms, local populations, old, traditional and improved varieties carrying a functional unit for heredity and possessing actual or potential value to science and practice (ITPGRFA, 2009).

Plant genetic resources provide the basis for sustainable agricultural production, adaptation to climate change, and economic development. Home gardens have allowed the adaptation and domestication of plants to extreme or specific ecological conditions, thus contributing to the diversification of cultivated plants. They provide opportunities to broaden the base of cultivated plant materials by underutilized crop plants and crop wild relative (Korpelainen, 2023).

The maintenance of plant genetic diversity through seeds of cultivated species and their wild relatives at national, regional and international levels, as well as promoting access to and equitable sharing of benefits arising from the use of these resources and associated traditional knowledge, is one of the objectives for FAO sustainable development goals (https://www.fao.org/sustainable-development-goals/indicators/251a/en/).

Fundamental and applied scientific research in the field of plant breeding in public, private and non-profit organizations nationally and internationally aim to reduce further biodiversity loss and respond of food demand under climate change scenarios. Achieving this one priority task is possible through the efficient use of genetic resources and traditional ecological knowledge about them. A stimulating factor is the provision of full-fledged free access to the gene pool stored in the gene banks, which also gives one a powerful long-term perspective on the effectiveness of the global conservation system plant biodiversity. (Engels and Ebert, 2021; Louafi et al., 2021).

Management of *ex situ* collections includes activities such as collection, research, evaluation, documentation and storage of plant germplasm, for the preservation of the diversity of species, appropriate use of their genetic potential selection, recovery in practice, full access and exchange (CBD, 2011). The main purpose of the genebanks is to represent the widest diversity of the gene pool and to make it free accessible (Dostatny et al., 2021).

The role of plant genetic resources stored in genebanks covers various effects on the environment and the socio-economic status of the population, both in rural and in the urban areas of the country. Landraces play an important role in the nutrition of people, as well as being a valuable donor of qualities to the breeding programs for the creation of modern varieties with high quality and high biological value (Villanueva et al., 2017). Identifying the link between climate change, agrobiodiversity, food security and healthy nutrition is the subject of comprehensive research activities (Muluneh, 2021). The global trend of reducing agrobiodiversity is a threat to the sustainability of agricultural production, respectively, and for the food chain. The role of home gardens as repositories of biological diversity has been acknowledged (Galluzzi et al., 2010; Vinatoru et al., 2019; Bratu et al., 2022).

The plant gene pool is a resource for creating innovations and finding new applications of existing crops and to develop improved varieties that provide food, feed, fuel, medicine and other raw materials for the world economy. After completing the acclimatization stage, the landraces are subjected to intensive breeding works, obtaining new genotypes with distinct phenotypic expressiveness (Negosanu et al., 2021; 2023; Bratu et al., 2023). Wild relatives are evaluated as potential sources of useful signs for the adaptation of crop plants to changing conditions (Mattana et al., 2021). The genetic variation in landraces caused by the specific factors of environment, shapes plant diversity (Bellon et al., 2015).

Landraces are an agri-food and historical-cultural heritage but are undergoing losses worldwide (Giupponi et al., 2021). Bulgaria is taking action to counteract this problem by following European guidelines. One of the important measures is the maintenance of National Register of local plant genetic resources.

The purpose of the study is to explore developing of conserved *ex situ* collections of Bulgarian landraces, preserved *on farm*, through new information strategies, supporting free access to plant gene pool in the context of their socio-economic significance.

MATERIALS AND METHODS

The National Programme of Conservation of Plant Genetic Resources for Food and Agriculture in Bulgaria has the following priorities: (1) conservation of cultural and historical heritage and present created values expressed in plant genetic resources for present and future generations and (2) contribution to national development, food safety, sustainable agriculture and agrobiodiversity management by means of conservation and utilisation of plant genetic resources for food and agriculture.

The National Seed Genebank at Institute of Plant Genetic Resources – Sadovo was established in 1984. The activities are carried out according to the FAO Genebank Standards (2014) for long-term conservation under controlled conditions.

The Information Centre was established in 1982 and it was renewed in 2021 under the BGPLANTNET project "Establishment of National Information Network GeneBank – Plant genetic resources", financed by the Bulgarian National Science Fund. The seed accessions are documented in an electronic database, according to the descriptors of FAO/Bioversity (2017). Passport information includes: catalogue number, taxonomic description, accession name, date of registration, biological status, donor and geographical origin. The database is organized as an electronic register which provides the ability to sort and analyse the status and the enrichment by descriptors.

During the period 1982-2023 collection missions for local horticultural varieties and populations were conducted to summarize the information available for the germplasm of local accessions collected from their typical growing regions, allowing their rational use in breeding and practice (Krasteva, 2007). Local farmers that preserve landraces *on farm* proved fundamental to create an inventory of traditional varieties and prevent the genetic erosion of landraces (Stoilova and Pereira, 2013). The landraces inventory was elaborated following the methodology of Maxted et al. (2019).

The study is using the survey method regarding the purpose of landraces cultivation in agricultural holdings by collecting accessions, interviewing farmers and analysing the passport data from conducted expeditions in rural areas.

Data were organized in Excel sheets with different fields: common name of the landraces, species, genus, family, elevation, latitude, longitude, municipality, region, farmer. Geographic coordinates and elevation were acquired through GPS from the farm/home garden of the private grower of the specific landrace. and database. The analysis was performed using MySQL software (Velcheva et al., 2023).

RESULTS AND DISCUSSIONS

Local accessions stored in the Bulgarian genebank collections are the result of the direct collection of seeds donated by farmers as a specific population, belonging to a certain locality and cultivated for traditional use. Due to its specific geographical location, diverse relief and climate features Bulgaria is distinguished by a rich botanical diversity that is used since ancients in traditional cuisine and local medicine.

Total amount of landraces registered in the passport database reached 11,063 (Table 1) and this figure is close to the real status of collections from expeditions (excluding not high number of duplications and some new accessions not yet registered in collection).

Table 1. Status of landraces in Bulgarian genebank collection (National Register, Date check 29.02.2024)

Crop groups	Number of accessions	% in the collection
Cereals	1,539	14 %
Grain legumes	2,907	26 %
Forages	1,668	15 %
Vegetable	3,951	36 %
Medical and aromatic plants	590	5 %
Others	408	4 %
Total	11,063	100 %

The largest collections are vegetables (36 %) and grain legumes (26%), followed by forages and cereals. Bulgarian genebank provides available passport data to the European web catalogue EURISCO (http://eurisco.ecpgr.org).

It was found that plant diversity grown in small holdings in Bulgaria is characterized by a large number of crops: vegetables, grain legumes, medicinal and aromatic species. The role of access to information on conserved plant biodiversity for achieving sustainable development of the regions can be considered in several directions – their participation in the agro-food chain, for production of traditional products, provide varietal diversity in local markets, as well as in rural tourism. Last but not least, local varieties have potential in relation to return of people to rural areas, hobby gardening and biological production. The role of access to gene pool information comes into focus on the biological, agricultural, social and economic prospects for use of these resources and traditional knowledge related to their cultivation, a time when society is sensitive to ecology and challenges, caused by climate change, with a growing need for higher yields and more well adapted crops. As a result of enrichment, collections were enriched with valuable landraces of genus *Phaseolus*, *Capsicum*, *Cucurbita*, *Allium, Lycopersicon*, etc. (Figure 1).

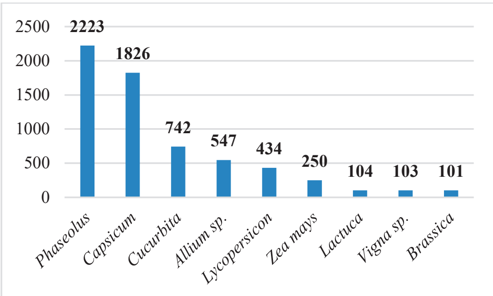

Figure 1. Number of collected landraces per crop

The highest percentage of *Phaseolus* collection (2083 acc. *Ph. vulgaris* L., 131 acc. *Ph. coccineus* L. and 9 acc. *Phaseolus* sp.) are collected from home gardens throughout mountains Rhodopes (700-1000 m), Balkan (500-650 m), Rila (700-1300 m), Pirin (700-900 m) and semi-mountain region of South and North Bulgaria (150-350 m). More than 100 accessions are collected from the villages near each of the region Smolyan, Velingrad, Devin, Samokov, Blagoevgrad, Troyan, Veliko Tarnovo, which makes these areas as hot spots of traditional *Phaseolus* varieties. Collected varieties of beans were identified, contributing greatly to the richness of this family at a national level. Likewise, 1825 acc. pepper (*Capsicum annuum* L.), 434 acc. of tomatoes (*Lycopersicon esculentum* Mill.), were collected from different parts of the country. This is linked to the climatic conditions and the dietary role of these plant genetic resources. Only the varieties that can develop at higher altitude such as Fabaceae can be found in Rhodopes, an almost totally mountainous region, while Trakya valley is rich in *Poaceae*, *Solanaceae* and *Cucurbita* landraces that can grow in the specific conditions. *Fabaceae*, *Solanaceae* and *Cucurbitaceae* together comprise 60% of all landraces. 80% of local beans are mostly preserved/grown in areas between 150 and 800 m. The sub-mountain and mountain areas are hotspots of traditional varieties due to anthropic and environmental factors (Figure 2).

Figure 2. Hot spots of landraces in mountain and semi-mountain areas in Bulgaria

Dobrudzha, historically considered one of the most important Bulgarian areas for wheat production, turned out to be very poor in *Poaceae* landraces, differently from most of the other regions of the country, probably due to the use of modern commercial varieties with the consequent loss of traditional cultivars. Strandzha, a hilly region characterized, has preserved many *Poaceae*, *Solanaceae*, and *Cucurbitacae* landraces thanks to the presence of traditional varieties. For example, the maize (*Zea mays*) landraces, despite being *Poaceae* crop, are good examples of conserved agro-ecotypes in home gardens. Plovdiv-Pazardzhik is the richest region in horticulture landraces, probably due to the climatic conditions and the unique logistics of the agri-food sector. The areas richest in number of landraces and in different crops cultivated as landraces were located inland in hilly and mountainous areas that are characterized by high environmental diversity. In these areas with a high level of agrobiodiversity are set *on farm* conservation of plant genetic resources were identified through a methodological approach using the criteria presence of landrace diversity and specific agroclimatic conditions, considering their additive advantages as local markets, rural tourism, consumers are ready to pay higher price for good taste of the traditional products. Landraces (*Fabaceae*, *Poaceae* and *Solanaceae*) are those containing landraces cultivated in a wide altitudinal range (from 0 to over 1300 m) but concentrated in sub-mountain areas (150-800 m). This is due to the fact that these families contain plants adaptable to, and grown in, these environments, such as beans (*Phaseolus* sp.), rye (*Secale cereale*), potatoes (*Solanum tuberosum*) and *Allium* sp. Landraces from *Fabaceae* family are cultivated in hilly, sub-mountain and mountain areas and 80% are local beans. Rye landraces are cultivated in hilly and sub-mountain areas. Potato landraces are mostly grown from 300 to 1200 m.

The particular environmental conditions of hilly and mountain areas, difficulties in communication, neglection, isolation, and traditional rural methods have guaranteed that varieties specifically adapted to those territories developed, becoming landraces as pulses, vegetables, cereals, forages, spices, etc.

The structure of farms from which seeds were collected from local varieties are different depending on the specifics of the area, but are generally small, aimed at satisfying the needs of the particular household. They are mainly used heirloom seeds that have been kept for many years in the family and are also livelihood for rural communities (Ulian et al., 2020). As a result of the enrichment and organized database of passport description of samples are created conditions for mapping the local gene pool, which is geographical, ecologically and culturally related to the region of cultivation. They exist justified prerequisites for expanding the expedition activity in specific regions of the country, rich in valuable plant resources - folk selection aimed mainly at selection for taste, fruit size, disease resistance. A high potential interest is revealed here connection with the achievement of a sustainable bioeconomy. The assessment of the value of plant genetic resources and their direct using local markets can contribute significantly to the conservation of agro-biodiversity while contributing to rural resilience communities (Balogh et al., 2016). Local varieties are a source of valuable qualities and biological characteristics that are preferred by consumers. The effective one however, a strategy for the protection of agrobiodiversity is closely related to the economic one value of products, therefore increasing attention to sustainable is needed foods, linking traditional agricultural productions with cultural and rural tourism (Brush et al., 1998). Awareness of the role of plant diversity in local farm will significantly increase the effectiveness of the strategy for its conservation.

Old local tomato varieties were well recognized in the past, but are now very poorly known, with the exception of the beef heart type ("Momino sarce", "Aleno sarce"). The most popular landraces of the Cucurbitaceae family grown in Bulgaria include "watermelon from Lubimetz", melon "Medena rosa" and pumpkin species type "Tsigulka" and "Big white". Minor crops - medicinal and aromatic plants, are very diverse and abundant in their natural habitats where the most abundant are species which have been cultivated for a long time, including chamomile, mint, lemon balm, valerian, thyme, and others.

In addition to the loss of genetic diversity, the significant loss of traditional knowledge on the use and growing of such varieties is evident. Thus, today only populations kept by poor households in marginal remote regions can survive, due to their inability to pay for commercial seed material. Families engage in food production for subsistence or small-scale marketing as well as knowledge related to agricultural practices is transmitted. Specific activities as *on farm* conservation could have a positive influence to the sustainability of the mountain and semi-mountain regions (Table 2).

Table 2. Indicators of conservation and reintroduction of landraces in mountain and semi-mountain areas

On farm as a conservation tool	*On farm* as a social catalyst	*On farm* as an income
• Sustainable management of resources • Environmental improvement • Local awareness about environmental and social problems • Biodiversity conservation • Investment in conservation and development of rural products	• Increased self-reliance, confidence and motivation for community development • Redefinition of local economy structure • Strengthening local cuisine, culture and heritage • Safety network for households with low incomes	• Migration of young people to villages • Local farmers economic benefits • Economic benefits to the villages • Some impacts on other economic sectors as tourism • Local community development projects

Small holdings make a significant contribution to crop production in a number of horticulture traditional varieties that are characterized by high labour intensity and a low level of mechanization. Small holdings make a significant contribution to the formation of rural communities and the sustainable development of rural areas. The data contained in the National Register reflect the situation of local plant diversity *ex situ/on farm* conservation and knowledge. As shown by this study, mountain and semi-mountain areas are hotspots of agrobiodiversity and a resource for sustainable, innovative, and quality agri-food chains.

The future perspectives to improve the protection and increase the free access to these valuable landraces:

- Improvement of cooperation between the sectors of agriculture and environment protection, which are responsible for the global biodiversity conservation in Bulgaria.
- Improvement of cooperation between the institutions involved in the plant genetic resources management and all users of the genebank potential.
- Support for creation of non-governmental organizations, which would be more involved in conservation of traditional local varieties.

- Development and financing of projects oriented to students' education in biodiversity preservation practises.

CONCLUSIONS

During last 40 years Bulgarian genebank has managed to collect a fairly large number of accessions of local and traditional varieties of diverse crops and their wild relatives. Although most of this material has been fairly well managed there are still gaps to be filled.

The study shows that at the current stage, despite the reduction of small holdings in the mountain and semi-mountain areas, they have a great socio-economic importance and role in the *on farm* conservation; support the vitality of hard-to-reach areas; play an essential role in supporting household incomes; have an essential role in increasing the sustainability of agriculture through the revival of specific local productions with added value, cultural and rural tourism, etc.

The datasets and analysis in this research will be useful to all stakeholders (civil society, farmers, and governance as well as researchers) as it provides information on distribution of Bulgarian landraces and can be trigger actions of their evaluation, characterization, access, and utilization of these resources.

In the future the National Register will be supplemented with details, describing conducted collection missions and that information will be made available via a web-enabled catalogue, part from Information System of Bulgarian genebank, following the recommendations of ECPGR (2021).

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EFFECT OF DROUGHT STRESS ON PROLINE AND CHLOROPHYLL CONTENTS IN SOME TOMATO GENOTYPES

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Abstract

Drought is the most important factor affecting yield loss in global agriculture. Drought stress negatively affects the physiological, genetic, biochemical, and morphological characteristics of plants. The objective of this study was to determine whether there are differential responses to drought stress on proline and chlorophyll content in some tomato genotypes. Drought deficit was induced by polyethylene-glycol (PEG 6000) solution using a control and two variants with different osmotic pressures (-2.72 Bars, -4.48 Bars). The determination of proline and chlorophyll content was evaluated after periods of 7/14 and 21 days after the induction of drought stress. Comparing the biological material studied, it can be observed that different concentrations of PEG 6000 influenced differently the analysed genotypes. High levels of proline content during drought stress were noticed also in Pontica, Viorica, Darsirius, and Buzau 47. The chlorophyll content of the leaves decreased proportionally with drought induction. The lowest chlorophyll content was recorded (22,965 SPAD units) after a longer period of water stress. The obtained results will be useful to serve in plant breeding programs.

***Key words**: tomato genotypes, proline and chlorophyll content, drought stress.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop widely spread all over the world and, too, this is a model plant of theoretical and practical significance in research (Arie et al., 2007; Guoting et al., 2020; Arshad et al., 2023; Dobrin et al., 2019).

In recent times, drought has occurred more frequently and more severely under the influence of global climate change. Most regions and countries are threatened by drought to varying degrees. (Fullana-Pericàs et al., 2018; Lehner et al., 2006; Lesk et al., 2016). Because drought is the most important factor affecting in global agriculture, drought stress may adversely affect growth and productivity, leading to final low fruit yields (Patanè et al., 2020, Liu et al., 2021). Water is an important factor to enhance the crop growth and productivity and it is essential for all living organisms including plants. Drought among various abiotic stresses, is one of the basic factors for restricting crops production (Vallivodan and Nguyen, 2006; Demidchik, 2018). It is predicted that one third of world population will be threatened by water shortage in 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). Among several physiological mechanisms developed by plants to counteract the adverse effects of drought, osmoregulatory factors, such as proline, are accumulated by plants to maintain the osmotic balance under stressful environments without damaging cellular activities (Isah, 2019). Practically, compatible osmolytes are potent osmo protectants that play a role in counteracting the effects of osmotic stress.

Proline is one of the most common compatible osmolytes in water-stressed plants. The accumulation of proline in dehydrated plants is caused both by activation of the biosynthesis of proline and by inactivation of the degradation of proline. The metabolism of proline is inhibited when proline accumulates during dehydration and it is activated when rehydration occurs (Isah, 2019; Yoshiba et al., 1997). A lot of data suggests a positive correlation between proline accumulation and plant stress (Shamsul Hayat, 2012). Proline may act as a signaling molecule able to activate multiple responses that are component of the adaptation process. Its accumulation in leaves and mainly in roots is considered a salt-sensitive trait in tomatoes, which can be used to select plants with different degrees of tolerance (Albino et al., 2002; Kavir et al., 2005). Detecting tomato plant drought stress is vital for optimizing irrigation and improving fruit quality. Moreover, there is a study it has demonstrated that proline acts as an osmo protectant and that overproduction of proline results in increased tolerance to osmotic stress. Genetically engineered crop plants that overproduce proline might acquire osmo tolerance, the ability to tolerate environmental stresses such as drought and high salinity. 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, et al., 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). Therefore, these parameters have been widely used in plant stress-tolerance physiology, crop breeding, and agronomy. Keeping in view of the increasing drought stress concerns upon tomato crop productivity, the objective of this study was to investigate whether there are differential responses to drought stress on proline and chlorophyll content in some tomato genotypes. The results obtained will be useful to serve in plant breeding programs for the development of tomato genotypes able to cope with actual and future climatic changes.

MATERIALS AND METHODS

The biological material used in this study was represented by six tomato genotypes (‘Buzau 47’, ‘Viorica’, ‘Pontica’, ‘Darsirius’, ‘Coralina’, ‘Carisma’). The experiment was conducted under normal (Vo - 0 Bars), and drought stress (V1 - 2.72 Bars, and V2 - 4.48 Bars) conditions created with Polyethylene-glycol (PEG6000) using the method suggested by Michael and Kaufman (1973). The plants were grown in culture pots filled with soil mixture. Plants were grown in greenhouse using a 14/10 h day/night photoperiod at 20/22ºC night/day temperature. Distilled water or PEG solution was added to each culture pot under normal and drought stress conditions, respectively, after every 2 days. The data for proline and chlorophyll contents was recorded on 7, 14 and 21 days after the induction of drought stress. Proline content was estimated according to the method proposed by Bates et al. [29] using L-proline as standard. A one-gram leaves sample was homogenised in 5 mL of aqueous sulfosalicylic acid (3%) and centrifuged at 14,000 g for 15′. Two mL of supernatant was added to 2 mL of mixed acetic acid and ninhydrin, and heated for 1 h at 100˚C. The mixture was cooled rapidly in an ice-bath and added to 4 mL of toluene. Absorbance was read in a spectrophotometer at 520 nm. Leaf chlorophyll content was measured by SPAD-502 portable chlorophyll meter (Konica Minolta, Osaka, Japan), a non-destructive method for measuring optical absorbance of chlorophyll (Sala, 2021). Measured values are expressed in SPAD units and there is a direct link between the total content of chlorophyll in the leaf and measured values. Data analysis was statistically performed using ANOVA and Tukey test for a three-way factorial design (Ciulca, 2006).

RESULTS AND DISCUSSIONS

Given the results in table 1, we can observe that the study genotypes exhibited different behaviours during the experiment. Thus, a decrease in chlorophyll content can be observed after a longer period of drought stress. A significant decrease was recorded after the 14-day period, followed by the 21-day period after stress induction (Table 1). Under drought conditions, reduction of chlorophyll content, a typical symptom of oxidative stress, may result from degradation of chlorophyll and photo-oxidation of the pigments.

Table 1. The influence of stress period on chlorophyll content (SPAD) in tomato

	Periods			
Genotypes	**7days**	**14days**	**21days**	**Mean G**
BUZAU 47	22.58 c	22.08 c	21.34 c	22.00 D
VIORICA	19.80 d	18.47 d	17.99 d	18.75 E
PONTICA	33.26 a	32.07 a	31.61 a	32.31 A
DARSIRIUS	27.04 b	25.24 b	24.90 b	25.73 B
CORALINA	26.33 b	24.15 b	24.20 b	24.90 C
CARISMA	19.08 d	18.29 d	17.75 d	18.37 F
Mean	24.68 X	23.38 Y	22.96 Y	23.68

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

Regarding the influence of PEG concentration on chlorophyll content, it can be seen from (Table 2) that the highest mean chlorophyll content was recorded for variant V0 (24.31 SPAD) and the lowest chlorophyll content was recorded for variant V1 (23.14 SPAD). Control variants have a SPAD value between 20.12 for Carisma and 31.27 for Pontica genotype. Treatments applied to induce drought stress resulted in significant reductions in chlorophyll content in proportion to the differences between these treatments. These results are in accordance with results obtained by (Tomescu D., 2015).

The genotypes studied showed mean values of this character between 18.37 SPAD in 'Carisma' and 32.31 SPAD in 'Pontica' genotypes. The largest decrease was found in 'Carisma' and 'Viorica' cultivars. These results showed that these two genotypes suffered a large decrease in their ability to capture light energy, which would have influenced their photosynthesis. The total chlorophyll reduction under drought conditions is thought to be related to the decrease in relative water content (Makbul et al., 2011). Osmotic adjustment can also be achieved by increasing the production of organic compounds (Sakya et al., 2018).

Table 2. The influence of PEG concentration on the chlorophyll content (SPAD)

	Drought stress			
Genotypes	**0 Bars**	**-2.72Bars**	**-4.48Bars**	**Mean G**
BUZAU 47	22.91 cd	20.77 d	22.32 cd	22.00 D
VIORICA	21.61 d	13.07 f	21.57 d	18.75 E
PONTICA	31.27 a	33.78 a	31.88 a	32.31 A
DARSIRIUS	23.15 c	29.75 b	24.28 b	25.73 B
CORALINA	26.80 b	24.35 c	23.53 bc	24.90 C
CARISMA	20.12 e	17.08 e	17.92 e	18.37 F
Mean V	24.31 X	23.14 Y	23.58 Y	23.68

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

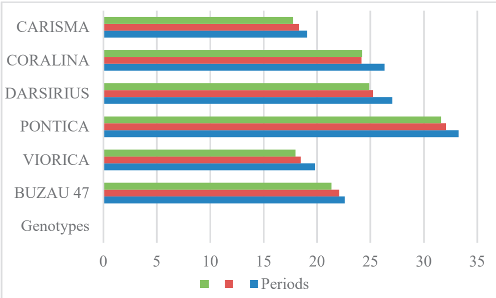

Figure 1. The influence of stress period on chlorophyll content (SPAD) in tomato

The proline content of the six genotypes under drought conditions was extremely diverse, ranging from 0.75 to 1.14 mg/g fresh weight (Table 3).

The proline content in 'Pontica' (1.14 mg/g) followed by 'Viorica' (1.13 mg/g) and 'Darsirius' (0.97 mg/g) genotypes were higher than others. This indicates that genotypes attempted to survive in drought conditions by increasing the proline content.

To survive in drought conditions, plants accumulate osmolyte compounds such as amino acids, proline, and organic acids (Behnamnia et al., 2009; Zhang et al., 2006; Ashraf et al., 2007; Hamim et al., 2009). However, not all plants exhibit osmotic adjustment through high proline accumulation.

Regarding the influence of stress period, a mean increase in proline content in tomato leaves can be observed from 0.91 mg/g fw after 7 days to 0.98 mg/g fw after 21 days (Table 3).

Table 3. The influence of stress period on proline content in tomato

	Period			
Genotypes	7 days	14 days	21 days	Mean G
BUZAU 47	0.83 c	0.84 c	0.86 c	0.84 C
VIORICA	1.10 a	1.12 a	1.16 a	1.13 A
PONTICA	1.08 a	1.13 a	1.21 a	1.14 A
DARSIRIUS	0.93 b	0.95 b	1.03 b	0.97 B
CORALINA	0.72 d	0.73 d	0.79 d	0.75 D
CARISMA	0.82 c	0.83 c	0.84 cd	0.83 C
Mean P	0.91 Z	0.94 Y	0.98 X	0.94

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

Regarding the influence of PEG concentration on proline content in tomato (Table 4, Figure 2), the means values were between 0.81 mg/g fw for variant V0 (0 Bars H_2O) and 1.08 mg/g fw for variant V2 (-4.48 Bars PEG6000).
We observed that leaf proline increased significantly under mild and severe drought stress compared with the control in all genotypes. The treatments applied to induce osmotic stress resulted in a significant increase in proline content in proportion to the differences between these treatments.
The same results were reported by another researcher (Mahlagha et al., 2012).

Table 4. The influence of PEG concentration on the proline content mg/g in tomato

Genotypes	Drought stress			
	0 Bars	-2.72Bars	-4.48Bars	Mean G
BUZAU 47	0.76 b	0.83 c	0.94 b	0.84 C
VIORICA	1.03 a	1.10 a	1.24 a	1.13 A
PONTICA	1.03 a	1.15 a	1.25 a	1.14 A
DARSIRIUS	0.65 c	1.01 b	1.25 a	0.97 B
CORALINA	0.67 c	0.74 d	0.84 c	0.75 D
CARISMA	0.73 b	0.83 c	0.93 b	0.83 C
Mean V	0.81 Z	0.94 Y	1.08 X	0.94

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

In tomatoes, the production and accumulation of proline are based on the duration of stress, genetic potential, and stress conditions (Jureková, 2011; Cooper et al., 2006). Among osmoprotectants, proline is an essential amino acid that has excellent antioxidant capabilities, helping to prevent cell death (Bhardwaj and Yadav, 2012; Oguz, et al., 2022).

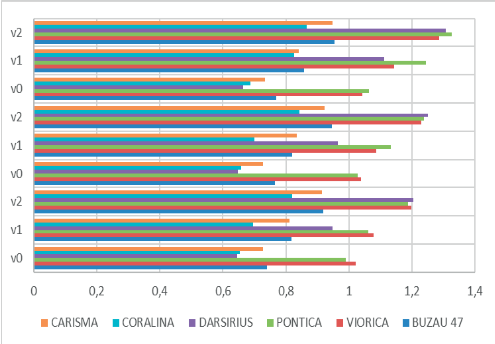

Figure 2. The influence of different drought stress treatments on proline content in tomato

Many scientists believe that the accumulation of proline due to stress serves as a biochemical indicator for the selection of resistant cultivars (Mwadzingeni et al., 2016).

CONCLUSIONS

Plant adaptive responses under drought, are mainly influenced by the timing, intensity, duration, and stress rate. Application of osmotic pressure caused significantly differences in terms of chlorophyll contents. Also, the biggest decrease was found in 'Carisma' and 'Viorica' cultivars. Treatments with PEG applied to induce osmotic stress determined significant increase of proline content in proportion to the differences between these treatments. The proline content in 'Pontica genotypes' was higher than others, this indicates that 'Pontica' followed by the 'Viorica' genotype attempted to survive in the drought conditions. The obtained results will be useful for tomato breeding programs, and also for seed production and tomato growers.

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PRE-TREATMENT OF SLOW-GERMINATING APIACEAE SEEDS FOR MICROGREENS

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Abstract

The purpose of the experiment is to test the application of different fertilizers, as growth regulators, for soaking slow-germinating seeds of the family Apiaceae, in the cultivation of microgreens. A potted laboratory experiment was prepared at the University of Forestry. Four fertilizers were tested: Bioforce, Humustim, Biotor, and Algreen, with four of the species of the Apiaceae family, suitable for growing microgreens: dill, carrot, parsley, and celery. Six variants were developed: the four fertilizers and two controls - with non-soaked seeds and with seeds soaked only in water. The seeds are soaked for 24 hours before sowing. Sowing is done in plates for microgreens, on a peat substrate. Of the four tested fertilizers as growth stimulators, Biotor shows the most complex results - it accelerates germination, affects the content of dry matter, increases the level of total sugars, and affects plastid pigments.

Key words*: Apiaceae, growth regulators, microgreens, soaked seed.*

INTRODUCTION

Micro-plants initially began to be grown as an eye-pleasing decoration and tasty addition to food. Later attention began to be paid to their nutritional and biological value and their effect on human health. Now they are gaining mass popularity as a healthy food.

Microgreens are young plants, other than sprouts, which have distinct stems, cotyledons, and the first very young true leaf. Compared to sprouts, which have germinated seeds ready in about 2-5 days and are eaten whole (along with the root), microgreens take longer (7-21 days) (Kumar et al., 2018).

The difference in time for obtaining micro-greens depends on the plant type. Vegetables, spices, herbs, etc. can be grown, and for most of them the harvest period is about 7-14 days, but there are slower-growing ones that need 21 days under optimal conditions (Stoleru et al., 2016; Kumar et al., 2018).

Stoleru et al. (2016) state that microgreens are sown at medium to high density and harvested shortly after the first true leaves appear, and according to Bulgari et al. (2021) the plants are harvested and cut above the roots.

Some of the most commonly used species are from the family Brassicaceae (broccoli, cauliflower, cabbage, turnips, radishes, arugula, etc.), Apiaceae (fennel, carrots, celery, parsley), Asteraceae (lettuce, chicory, etc.), Cucurbitaceae (melon, cucumber, pumpkin), Fabaceae (beans, chickpeas, broad beans, lentils, peas, etc.) etc. In addition to families, microplants can be divided according to their color (yellow, green, red, purple, or variegated), and aromatic species can have a very strong smell (Saleh et al., 2022).

Microgreens from the Apiaceae family are quite aromatic and rich in oils and resins, which give them specific flavors. Dill is used to flavor many dishes, but it also has therapeutic properties against inflammations, and microbial infections, for relieving insomnia, etc. (Giordano et al., 2022).

Fabek Uher et al. (2023) found that when dill, red cabbage, and alfalfa were grown as micro-greens, in hydroponic technology on perlite, with the highest levels of total carotenoids (0.196 mg/g tw), total chlorophyll (0.713 mg/g tw) and chlorophyll a (0.528 mg/g fw) were detected in dill. It has also shown the highest levels of the other investigated components: dry matter, ascorbic acid, flavonoids, etc.

The type of plants is the amount and type of carotenoids contained in them. For example, pigmented vegetables such as salad beets and carrots are rich in lycopene and beta-carotene, while green plants, such as spinach and cabbage, are rich in lutein and zeaxanthin (Saltveit, 2010, L. Yadav, 2019).

Different types of seeds require different times to sow and germinate. Some seeds require soaking, others weighting or covering with soil. (Bulgari et al., 2021).
Soaking seeds in cold water is recommended for some large-seeded crops, including peas and radishes, according to Lee. Fertilizers mixed into the growing substrate or applied after emergence through soluble fertilizers have been found to result in rapid growth and a high yield of microgreens. According to Kou, 10 ml of calcium chloride solution applied daily for 10 days resulted in an increase in the biomass of cruciferous vegetables compared to water (Li et al., 2021).
The purpose of the experiment is to test the application of different fertilizers, as growth regulators, for soaking slow-germinating seeds of the family Apiaceae, when growing them as microgreens.

MATERIALS AND METHODS

The experiment was carried out at the University of Forestry, Sofia, Bulgaria, in 2023.
For the experiment, seeds of the following species from the Apiaceae family were used for the production of microgreens: Dill (germination period 4-5 days); Carrot (germination period 4-7 days); Parsley (germination period 7-10 days); Celery (germination period 10-14 days). The organic fertilizers used in the experimental part, according to the manufacturers, have the following content:
Bioforce - Improves the germination of seeds, stimulates the development of the root system, and increases the resistance of plants against stress (Table 1).

Table1. Mineral and organic content of Bioforce

pH						
H_2O	KCL	∑ N-NH_4+NH_3 mg/kg	P_2O_5 mg/ 100 g	K_2O mg/100 g	Hummus %	
9.0	8.4	155.5	260	760.3	9.0	
Total micronutrient content						
N %	P %	K %	Ca %	Mg %	N %	
0.94	0.61	0.74	2.21	0.74	0.94	
Chemical analysis of "mobile" elements in EDTA						
Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg			
1.5	1720	42.8	6.2			
Total forms of heavy metals						
Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Ni mg/kg	Pb mg/kg	Cd mg/kg
114	30	361	8400	14	under 6	under 2

Biotor Ruse - Organic fertilizer from red California worm, rich in useful microorganisms, enzymes, vitamins, and amino acids (Table 2).

Table 2. Mineral and organic content of Biotor Ruse

pH	Organic %	Total N mg/l	Total P mg/l	Total K mg/l	Mg mg/l
7.31	32.33	625	381.93	976.27	550.12

Humustim (potassium humate) is a growth regulator that controls the metabolism of plants and stimulates their growth and development (Table 3).

Table3. Mineral and organic content of Humustim

Total N %	P_2O_5 %	K_2O %	CaO %	MgO %	Organic C %	Organic %
2.0	1.0	6.5	2.0	0.5	23.0	45.0

Algreen - organic fertilizer, concentrated extract of fresh algae containing natural phytohormones (PGR), alginic acid, Nitrogen (N), Sulfur (S), Magnesium (Mg), and trace elements in chelated form (Table 4).

Table4. Mineral and organic content of Algreen

N g/l	S g/l	MgO g/l	B g/l	Mo g/l	Alginic acid g/l	Natural plant hormones	Organic g/l	pH
60	70	60	5	2.6	40	> 300 ppm	20	6.5-7.5

Plastic working trays with a size of 15.3 x 10.5 - approximately 161 cm^2 were used.
The used nutrient substrate "Terranovo" is the same for all variants – it is a mix of compost, coconut fiber, and perlite, with a pH of 5.5-6.5 according to the manufacturer's data (Agroflora S.A.).
Six variants have been developed. In five of them, the seeds are soaked – with water or in solutions of the selected nutrient media, and there is one without soaking - a control for comparison. The six variants are as follows:
1) control, untreated seeds (NT);
2) water treatment (WT);
3) treatment with Bioforce (BF);
4) treatment with Humustim (HT);
5) treatment with Biotor (BT);
6) treatment with Algreen (AL).

Only of the selected nutrient media, has recommendations for soaking the seeds, in which approximate norms for preparing a solution for soaking seeds are proposed. The concentration used for variants BF, HT, and BT is normally 50 ml/2 l of water. For the last treatment, because the nutrient medium is based on a concentrated extract of fresh algae, and in this case, another concentration is used - 10 ml/10 l of water.

The recommendation for Apiaceae family seeds is to soak them for nearly two days. For the experiment, they were soaked in the prepared solutions for 24 hours. After that, they are filtered, dried, and sown in the previously prepared plates, with three replications. Two sowing dates were made, and the sowings were carried out in stages: 04/04/2023 - sowing of dill and carrot; 04/28/2023 - sowing of celery and parsley.

Seeding rates are calculated according to seed size and production container size and recommendation by the seed supplier – for the celery - 0.3 g, parsley and carrot - 0.8 g each, and dill - 1 g.

The number of days until seed germination was monitored, and 20 seeds of each variant were placed in petri dishes, on a humidified medium. Counting was done on the 4th and 10th day for seed germination by variants and for plant emergence, by variants and replicates. Reported percentages of germinated plants are shown as mean per variant.

Chlorophyll content: by determining chlorophylls and carotenoids in laboratory conditions according to the method of Vernon L. P. (1960), to determine the photosynthetic activity of plants; Sugar content: percentage content of sugars in the young plants (Brix, %) with a refractometer, model - Digital refractometer 32145, manufactured by B & C Germany; Dry matter content: From each variant, 50 plants were cut to determine fresh weight (FW) and dry weight (DW). After measuring the fresh weight, the samples were dried in an air dryer at 60°C until a constant dry weight was obtained. Then the data were recalculated to percent dry weight (DW × 100/FW).

RESULTS AND DISCUSSIONS

During the performance of the experiment, the temperature and humidity in the working room were monitored because it was not heated.

In the month of April, the temperature in the room ranges between 11-15°C, and a peak of 22°C was measured. On that day, the lowest atmospheric humidity for the month was measured in the room, 80%. During the rest of the time, the humidity is in the range of 90-95%

These two factors are important for creating optimal conditions for plant germination and emergence, and in this case, they slowed down the development of the first two species tested for microgreens - dill and parsley (Figure 1).

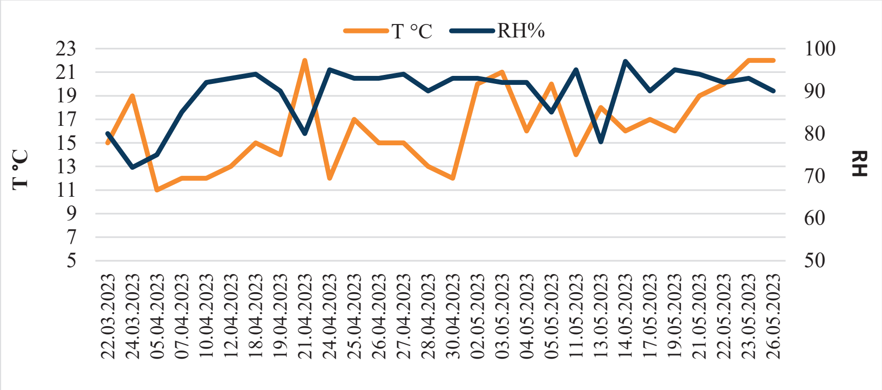

Figure 1. Temperature and air humidity in the working room

Phenology observations

Dill and carrot. In the first experiment, on the 4th day after the treatment of the seeds, the beginning of germination was reported for the dill, with the option of soaking with BT. The carrot had no sprouted seeds. On the 10th day, mass germination was observed in both species and the case of dill, the non-soaked seeds surpassed the soaked ones (Table 5).

Table 5. Seed germination and plant emergence (%) for dill and carrot

Variant	Dill			Carrots		
	Seeds		Plants	Seeds		Plants
	4th day	10th day	10th day	4th day	10th day	10th day
NT	0%	90%	60%	0%	80%	37%
WT	0%	80%	53%	0%	85%	38%
BF	0%	60%	48%	0%	75%	55%
HT	0%	70%	60%	0%	70%	50%
BT	(~5%)	80%	53%	0%	80%	50%
AL	0%	85%	42%	0%	85%	52%

On the 10th day, plant germination was recorded for all variants, with the control and the variant with BF for dill ahead of the others in %. In the case of carrots, on the 10th day, four of the variants have an average of 50% and over 50% germination of the plants (Table 5).

Celery and parsley. The second sowing was carried out with celery and parsley and, unlike the development of the previous two crops, a significant difference was found between those at the beginning. On the fourth day, the celery has no germinated seeds. This was registered on the 10th day – 10% in two of the options: soaking with BF and treating the seeds with BT (Table 6).

Table 6. Seed germination and plant emergence (%) for celery and parsley

Variant	Celery			Parsley		
	Seeds		Plants	Seeds		Plants
	4th day	10th day	10th day	4th day	10th day	10th day
NT	0%	0%	0%	0%	67%	57%
WT	0%	<10%	0%	10%	50%	70%
BF	0%	10%	0%	50%	90%	20%
HT	0%	<10%	0%	45%	90%	20%
BT	0%	10%	<10%	60%	90%	90%
AL	0%	20%	<10%	55%	95%	53%

In the last variant - soaking with AL, 20% of germinated plants were recorded. In the control variant, without soaking, there was no germination even on the 10th day. On the 10th day, germination of the seeds in the working trays was also reported. In the case of celery, there is a beginning of germination by soaking the seeds with BT and AL (Table 6).

In parsley, on the 4th day, the seeds germinated was reported. On the 10th day, in working trays almost complete germination was recorded in the variant with BT, while in the variant BF and HT the germination was weaker (Table 6).

From the indicated data, it follows that the treatment of seeds with the BT accelerates and improves germination. The treatment of seeds with fertilizer with AL, WT, BF, and HT is ordered. According to the germination results of the plants in the working trays, the first biofertilizer (BF) outperformed the water-treated seeds in some cases.

Dry matter in %

In dill, the dry matter had the highest percentage in the second BT (3.6%), followed by the variant treated with AL (3.2%). The lowest (1.4%) was measured in the variant by soaking the seeds in water only (Figure 2).

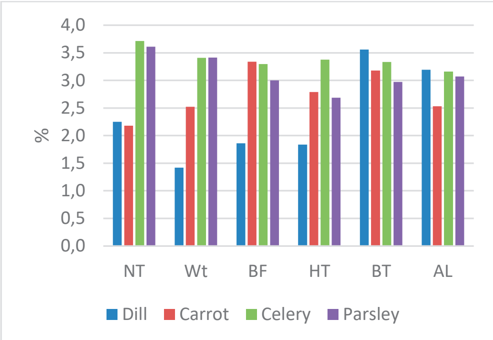

Figure 2. Dry matter (%)

The microgreens of celery had a higher dry matter content. It had approximately the same amounts for the individual variants, and the difference between the highest value (NT) and the lowest (AL) is about 0.5%. The dry matter of parsley is within the limits of around and above 3%. The highest percentage is the NT (3.6%), followed by the treating the seeds in WT (3.4%). The percentage (2.7%) is the lowest in the variant of treated seeds with HT. (Figure 2).

Sugar content % Brix

Parsley and celery microgreens showed a higher percentage of total sugars in the untreated seeds, compared to the treated variants. In the variant with soaking the seeds only in water, the percentage of total sugars also increased in the other two crops - dill and carrot. Dill was affected by seed treatment with the BF, although the values were not very high, while parsley had the lowest percentage. Treatment with HT in dill increased the content of sugars and was the highest of the tested crops. With the BT seed treatment, the percentage content of all crops was almost equalized, without a strong increase or decrease, but dill and carrot did better (Figure 3).

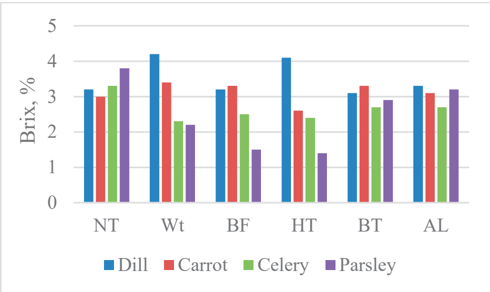

Figure 3. Content of total sugars in %

All types of microgreens had a content of total sugars above 2.5%, In the different variants, there are more pronounced peaks, and there are species with lower and higher sugar content.

Chlorophyll and carotenoid content

Compared to mature vegetables, microgreens have a higher content of vitamins and carotenoids. Dill microgreens showed the best photosynthetic activity of all plants and the level of chlorophyll A (Figure 4).

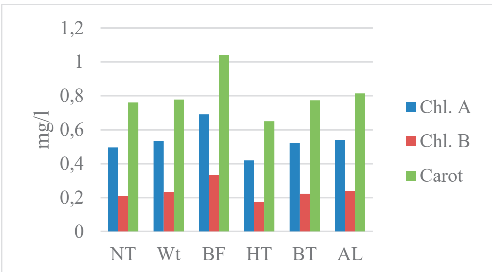

Figure 4. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in dill

It ranged from 0.419 to 0.691 mg/l, with the best performance being the variant with the BF seed treatment and the lowest for the seeds, treated with HT. The carotenoid content of dill ranges from 0.650 to 1.040 mg/l. The remaining four variants also have good indicators - treatment of seeds with AL, BT, and the control with untreated seeds, and only those treated with humate fertilizers have a lower content than the control (Figure 4).

The best photosynthetic activity in carrots was shown by plants from the control variant - without seed soaking, with the content of chlorophyll A reaching 1.982 mg/l. In all variants with seed treatment, it is weaker, compared to the control, with vitamin A content ranging from 0.281 to 0.577 mg/l. The values are lowest when plants are treated with BF Carotenoid values in carrots ranged between 0.455 and 1.982 mg/l, being the highest in the control variant without seed soaking. In all variants with seed soaking, they were lower compared to the control and ranged from 0.455 to 0.871 mg/l (Figure 5).

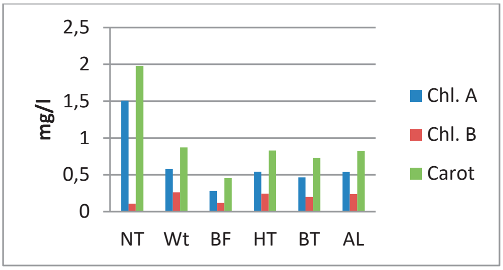

Figure 5. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in carrot

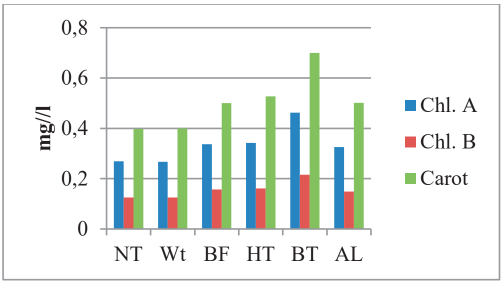

Figure 6. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in celery

Celery showed lower physiological activity in the NT and the variant with WT compared to the treated variants. and the level of chlorophyll A in it ranged from 0.267 to 0.462 mg/l, being the highest in the treated variant with BT. The

levels of carotenoids in this variant range between 0.400 and 0.700 mg/l, and after the variant is treated with BT is the variant HT (Figure 6).
Parsley photosynthetic activity is better and in three of the variants, the values are almost equal. Chlorophyll A levels ranged from 0.299 to 0.535 mg/l, with the highest values, similar to carrot, being the untreated control (Figure 7).

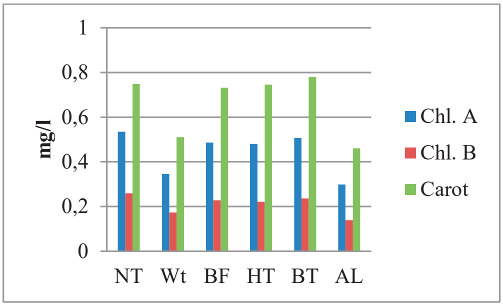

Figure 7. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in parsley

With almost equal values, are the variants treated with BF and HT. The level of carotenoids in parsley is also higher, ranging from 0.461 to 0.780 mg/l. The variant with soaked seeds in the BT has the highest values, closely followed by the NT, HT, and BF (Figure 7).
Photosynthetically active chlorophyll A in all variants ranges from 0.256 to 0.691 mg/l, and the ratio between chlorophyll A and chlorophyll B (1:2) is respected and is an indicator of good photosynthetic activity. Data for carotenoids show higher values and are in the range of 0.400 - 1.040 mg/l.

CONCLUSIONS

Three of the nutrient media used to lead to an acceleration of seed germination and plant emergence – first is treatment with Biofertilizer, followed by organic fertilizer with algae extract Algreen and biofertilizer Bioforce. Good results are also obtained by just soaking the seeds in water.
Soaking the seeds in Biofertilizer, Bioforce or Algreen organic fertilizer affects the dry matter content of the microgreens. When treating the seeds with Biotor, all types of microplants have a content of total sugars above 2.5%.
Both - Bioforce and Biotor - have an impact on the accumulation of chlorophyll and carotenoids.
Dill and carrots have a higher content of total sugars than the others. Carrot, dill, and parsley are distinguished by a higher content of carotenoids.

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PHYTOTOXICITY EFFECTS OF LEAD ON SEEDS GERMINATION AND SEEDLING GROWTH OF `WIZARD ROSE` AND `WIZARD JADE` COLEUS VARIETIES

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Abstract

The study presents the comparative analysis on the impact of different concentrations of Pb(II) (25 to 300 mg/kg) on germination, seedling rate, velocity, tolerance index, toxicity index and vigour index at Coleus blumei 'Wizard Rose' and 'Wizard Jade'. The experience was conducted in 6 variants with 3 replicates, each replicate having 10 seeds. The humidity of the substrate was performed with water for the control sample (C) and with $PbCl_2$ solutions for the other 5 variants. At high concentrations of lead (300mg/l), a significant decrease in the germination percentage can be seen of approximately 20% in the case of the 'Wizard Rose' coleus and 50% in the case of the 'Wizard Jade' coleus. The results obtained in the study show that lead had the highest inhibitory effect on germination, seedling rate, velocity, and plant growth at the coleus 'Wizard Jade'. The degree tolerance of Coleus blumei varieties to the stress caused by the lead decreases with the increase in the concentration of Pb(II), coleus 'Wizard Rose' highlighting a better tolerance to Pb(II) compared to coleus 'Wizard Jade'.

Key words: *Coleus blumei, germination, toxicity, velocity.*

INTRODUCTION

At present, various activities such as industrialization and urbanization contribute to soil contamination with various heavy metals, Hadi & Aziz, 2015. In recent decades, worldwide soil pollution with heavy metals is one of the major problems for both environment and human health (Singh et al., 2011, Bihola et al., 2020). Among the potential heavy metals that are neither essential elements nor have any role in the process of plant cell metabolism, lead (Pb) is a highly toxic and persistent environmental pollutant derived from various sources (Sharma & Dubey, 2005), easily absorbed and accumulated in different parts of a plant, Hadi & Aziz, 2015. Lead is one of the most prevalent heavy metal contaminants in soil that can be retained for a long period of time ranging from 150-5000 years (Prasad et al., 1999). Although lead has no biological purpose, it can disrupt the morphology, physiology and biochemistry of a plant (Fahr et al., 2013; Dabgar et al., 2023). The effects of heavy metals on seed germination differ from species to species due to differences that occur in seed structure, especially in seed layers that not only have a different chemical composition but also a wide range of anatomical shapes that do not exist in any other organ or plant tissue (Yuan et al., 2013; Islam et al., 2008). In general seed germination is dependent on the type of metal and its concentration. Over the years, numerous studies have been conducted focusing on the effect of heavy metals on seed germination and seedling growth (Hatamzadeh et al., 2012; Fatarna et al., 2017). Seed germination represent the starting point in the life cycle of a plant and is considered the earliest stage of a plant's exposure to heavy metal stress, so detection of seed germination and seedling growth index is a good method to evaluate the capacity of plants to heavy metal toxicity (Bae et al., 2016; Taghizadeh & Solgi, 2017). Pb inhibits seed germination and delays seedling growth, decreases germination percentage, germination index, root and shoot length, tolerance index and dry matter content of roots and sprouts (Mishra et al., 2006; Hadi & Aziz, 2015; Dabgar et al., 2023; Stratu & Costică, 2013). The results of studies presented in the literature indicate lower concentrations of heavy metals (between 3 and 33 ppm) with partial inhibitory effect on seed germination in *Picea abies* L. while higher concentrations (100 ppm) of all metals cause partial inhibition in some species (Hagiu Zaleschi et al., 2022; Malkowski et al., 2002; Jeliazkova, 2000) while in others total inhibition. High Pb concentrations inhibit plant growth development which leads to reduction of fresh biomass, tolerance index in roots, sprouts and leaves (Cozma et al., 2019). Similar results have been obtained in other studies at increased Pb concentrations: root, sprouts and leaf growth; fresh and dry biomass is much reduced in *Lavandula angustifolia* (Hagiu Zaleschi et al., 2022), in *Zea mays* (Cimrin et al., 2007), in *Ipomoea aquatica* (Göthberg et al., 2004), in *Ageratum houstonianum* Mill (Stratu & Costică, 2013) in mung bean genotypes (Hassan & Mansoor, 2014).

MATERIALS AND METHODS

For the experiment, the biological material used was professional seeds of *Coleus blumei* 'Wizard Rose' and 'Wizard Jade' purchased from Anthesis International S.R.L.

The experiment was carried out at the Horticultural Research Centre and at the Greenhouses of the ICAM Research Centre of “Ion Ionescu de la Brad” Iasi University of Life Sciences, Romania. The methodology has been developed in line with the OECD guidelines for testing of chemicals.

The experiment was organized in six variants (in the first variant the substrate was uncontaminated and was considered the control variant V_1-control, and in the other five variants the substrate was contaminated with Pb(II) ($PbCl_2$): V_2 - 25 mg/kg, V_3 - 50 mg/kg, V_4 - 100 mg/kg, V_5 - 250 mg/kg, V_6 - 300 mg/kg) of three replicates, each variant having 10 seeds. Substrate contamination was carried out before filling the containers, in order to obtain the established concentration of lead, the amount of $PbCl_2$ required to contaminate the substrate was calculated for each variant.

After lead application, the substrate was allowed to stand for 3 days for efficient homogenization. Sowing was carried out in 2 kg containers in substrate consisting of two parts peat and one part garden soil. Germination testing was carried out under greenhouse conditions at temperature: 22ºC ± 10ºC; humidity: 70% ± 25% and photoperiod: minimum light of 16 hours. For each pot watering was done with tap water applying the same amount of water per container. Measurements were carried out daily at the same time until three successive measurements showed the same values. Measurements were made on the influence of lead on germination percentage, seedling rate, velocity, toxicity index, tolerance index, and vigour index.

RESULTS AND DISCUSSIONS

Germination monitoring was carried out daily at the same time until three consecutive measurements showed the same values. In each experimental variant, the germination process was carried out by averaging the three replicates. The results obtained in each experimental variant contaminated with lead were compared with the control sample. The seeds of 'Wizard Rose' and 'Wizard Jade' used in the experiment started to germinate 7 days after sowing, a process that took place over 8 days from the start (Table 1).

Table 1. Influence of lead on the seed germination of the 'Wizard Rose' and 'Wizard Jade'

Variant		Sowing date	Germination onset date	End of germination date	Total germination *
V_1 (control)	'Wizard Rose'	8.04.2021	14.04	20.04	98
	'Wizard Jade'		14.04	21.04	96
V_2	'Wizard Rose'	8.04.2021	14.04	20.04	96
	'Wizard Jade'		14.04	21.04	88
V_3	'Wizard Rose'	8.04.2021	14.04	21.04	92
	'Wizard Jade'		15.04	21.04	80
V_4	'Wizard Rose'	8.04.2021	15.04	21.04	84
	'Wizard Jade'		15.04	21.04	78
V_5	'Wizard Rose'	8.04.2021	15.04	21.04	80
	'Wizard Jade'		15.04	21.04	60
V_6	'Wizard Rose'	8.04.2021	15.04	21.04	78
	'Wizard Jade'		15.04	21.04	48

The results on the influence of Pb(II) on germination, seedling rate, velocity, toxicity index, tolerance index and vigour index in the two coleus varieties are shown in figures 1-8. From the analysis of the data on the influence of Pb(II) on germination rate in 'Wizard Rose' and 'Wizard Jade' it is evident that both germination rate, relative germination index and vigour index decrease with increasing heavy metal concentrations from 250 mg/kg to 300 mg/kg.

Regarding the germination degree of the seeds of the two coleus cultivars, it can be observed that 'Wizard Jade' is more affected by lead toxicity in the germination substrate compared to 'Wizard Rose' in all experimental variants (Figure 1). In both varieties, a decrease in germination percentage with increasing Pb(II) concentrations in the substrate is evident compared to the control, by 20% in 'Wizard Rose' and 50% in 'Wizard Jade'. In 'Wizard Rose' the value of this indicator decreases from 98% for the control sample to 78% for 300 mg/kg Pb(II). In the case of 'Wizard Jade', at the same concentration value for Pb(II), the germination rate decreases compared to the control and registers in the case of the contaminated variant with the highest concentration the value of 48%.

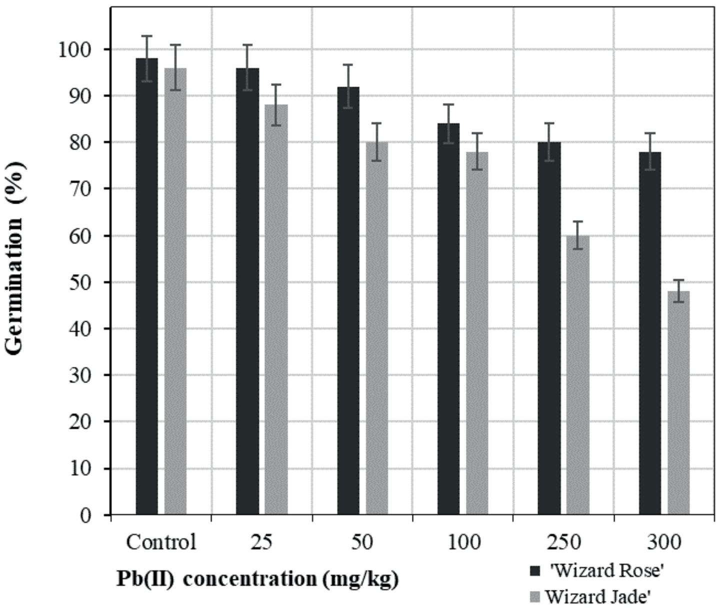

Figure 1. Influence of Pb(II) stress on the germination (%)'Wizard Rose' and 'Wizard Jade' cultivars

The effect of lead on germination was evidenced by the analysis of the main germination and sprouting indicators: sprouting dynamics, sprouting rate and sprouting speed.

The results of the dynamics of plant sprouting in the two coleus varieties are presented in Table 2. For both species, sprouting started 7 days after sowing and took place over six days in the control variants and eight days in the other experimental variants. In 'Wizard Rose' under lead-contaminated conditions the earliest sprouting occurred 7 days after sowing in the variants with lead concentrations of 25 mg/kg soil and 50 mg/kg soil. In the variants where lead was present at concentrations of 100 mg/kg soil (V_4), 250 mg/kg soil (V_5) and 300 mg/kg soil (V_6) sprouting started 8 days after sowing and took place over 8 days. Compared to the control, the increase of lead concentrations in the substrate resulted in a minor delay in plant sprouting (1 day).

In the case of 'Wizard Jade', the sprouting started 7 days after sowing in the control variant and the variant with the lowest Pb(II) concentration and 8 days after sowing in the other experimental variants. It is worth noting that in the case of the other variety, in the variants with the highest lead concentrations (V_5, V_6) the sprouting occurred within 8 days.

Table 2. The dynamic emergence of 'Wizard Rose' and 'Wizard Jade' cultivars

Variant		Date/sprouted plants (%)							
		14.04	15.04	16.04	17.04	18.04	19.04	20.0	21.04
V_1 (control)	'Wizard Rose'	25	49	82	83	90	98	98	98
	'Wizard Jade'	26	36	75	76	80	93	95	96
V_2	'Wizard Rose'	25	44	70	82	87	90	96	96
	'Wizard Jade'	25	39	48	57	60	68	76	88
V_3	'Wizard Rose'	20	25	46	55	67	76	88	92
	'Wizard Jade'	0	18	32	49	62	68	77	80
V_4	'Wizard Rose'	0	15	31	48	60	68	81	84
	'Wizard Jade'	0	16	37	44	58	69	75	78
V_5	'Wizard Rose'	0	10	31	45	65	78	79	80
	'Wizard Jade'	0	12	28	33	40	48	53	60
V_6	'Wizard Rose'	0	10	19	49	56	66	73	78
	'Wizard Jade'	0	5	10	23	33	41	45	48

The dynamics of the germination rate in the two coleus varieties (Table 3) show some variations determined, in particular, by the concentration of lead in the substrate. In both varieties, in the control variants the absence of lead in the substrate resulted in maximum values of germination rate in the first three days, 33% in cultivar 'Wizard Rose' and 39% in cultivar 'Wizard Jade'.

Seeds sown in substrate contaminated with 25 mg/kg Pb(II) soil had a higher germination rate during the first three days after the start of sprouting, (10-39% in 'Wizard Rose' and 19-26% in 'Wizard Jade') and on the eleventh day the lowest rate in both cultivars (5% in 'Wizard Rose' and 3% in 'Wizard Jade'). Variants with substrate contaminated with Pb(II) in concentrations of 250 mg/kg soil and 300 mg/kg soil (V_5-V_6) had high germination rate values on the first days, with maximum values the tenth day after germination onset, after which they were decreasing. In both varieties, lead contamination resulted in a lower germination rate compared to the control, indicating slower and more inuniform germination. In 'Wizard Rose' maximum values were recorded on the first day after the onset of sprouting in variants V_2-V_3, on the second day in variants V_4-V_5 and on the third day after the onset of sprouting in variant V_6. In 'Wizard Jade' maximum values were recorded on the first day after the onset of sprouting in variants V_1, V_2, on the second day in variants V_3-V_4 and on the third day after the onset of sprouting in variants V_5, V_6. The highest germination rate was observed in the control variants three days after the onset of sprouting (33% 'Wizard Rose', 39% 'Wizard Jade'). In the case of these variants, where the total percentage of germinated seeds was above 96%, on the penultimate day of measurements the germination rate dropped suddenly to low values.

Germination velocity (Table 4) was unfavourably influenced by increasing Pb(II) concentration in the substrate. The velocity showed different values from variety to variety, from variant to variant, and during the sprouting period. In both varieties, the control variants, with seeds sown on substrate uncontaminated with Pb(II), the highest values of velocity (3.67% in 'Wizard Rose 'and 4.33% in 'Wizard Jade') were recorded nine days after the end of the experiment respectively the third day after the onset of sprouting. The evolution of the two varieties in the case of seeds contaminated with Pb(II) (V_2-V_6) was characterized both by larger differences determined by the resistance of the variety to Pb(II) , and by fluctuations from day to day, with alternating maxima and minima.

Thus, germination in 'Wizard Rose' in the control variant was more rapid in the first three days, with maximum velocity showing values between 3.00% and 3.67%, and low velocity in the last days, with values reaching 0.67%. In 'Wizard Jade', the velocity results also showed maximum values in the first three days and a more pronounced decrease in values compared to the other variety nine days after sowing. Increasing the concentration of Pb(II) in the germination substrate to 300 mg/kg substrate (V_6 variants) resulted in a decrease of the velocity, which at the beginning of sprouting was 1.25 in cultivar 'Wizard Rose' and 0.63% in cultivar 'Wizard Jade', and towards the end of the period 0.36 in cultivar 'Wizard Rose' and 0.21% in cultivar 'Wizard Jade'.

Table 3. The sprouting rate of the 'Wizard Rose' and 'Wizard Jade' cultivars under exposure to different doses of $PbCl_2$

Variant		**Date/ sprouting rate %**							
		14.04	**15.04**	**16.04**	**17.04**	**18.04**	**19.04**	**20.04**	**21.04**
V_1 (control)	*'Wizard Rose'*	25	24	33	1	7	8	0	0
	'Wizard Jade'	26	10	39	1	4	13	2	1
V_2	*'Wizard Rose'*	25	19	26	12	5	3	6	0
	'Wizard Jade'	25	14	9	9	3	8	8	12
V_3	*'Wizard Rose'*	20	5	21	9	12	9	12	4
	'Wizard Jade'	0	18	14	17	13	6	9	3
V_4	*'Wizard Rose'*	0	15	16	17	12	8	13	3
	'Wizard Jade'	0	16	21	7	14	11	6	3
V_5	*'Wizard Rose'*	0	10	21	14	20	13	1	1
	'Wizard Jade'	0	12	16	5	7	8	5	7
V_6	*'Wizard Rose'*	0	10	9	30	7	10	7	5
	'Wizard Jade'	0	5	5	13	10	8	4	3

Table 4. The velocity of the 'Wizard Rose' and 'Wizard Jade' cultivars under exposure to different doses of $PbCl_2$

Variant		**Date/ velocity (%)**							
		14.04	**15.04**	**16.04**	**17.04**	**18.04**	**19.04**	**20.04**	**21.04**
V_1 (control)	'Wizard Rose'	3.57	3.00	3.67	0.1	0.64	0.67	0.00	0.00
	'Wizard Jade'	3.71	1.25	4.33	0.1	0.36	1.08	0.15	0.07
V_2	'Wizard Rose'	3.57	2.38	2.89	1.2	0.45	0.25	0.46	0.00
	'Wizard Jade'	3.57	1.75	1.00	0.9	0.27	0.67	0.62	0.86
V_3	'Wizard Rose'	2.86	0.63	2.33	0.9	1.09	0.75	0.92	0.29
	'Wizard Jade'	0.00	2.25	1.56	1.7	1.18	0.50	0.69	0.21
V_4	'Wizard Rose'	0.00	1.88	1.78	1.7	1.09	0.67	1.00	0.21
	'Wizard Jade'	0.00	2.00	2.33	0.7	1.27	0.92	0.46	0.21
V_5	'Wizard Rose'	0.00	1.25	2.33	1.4	1.82	1.08	0.08	0.07
	'Wizard Jade'	0.00	1.50	1.78	0.5	0.64	0.67	0.38	0.50
V_6	'Wizard Rose'	0.00	1.25	1.00	3	0.64	0.83	0.54	0.36
	'Wizard Jade'	0.00	0.63	0.56	1.3	0.91	0.67	0.31	0.21

Influence of Pb(II) on germ development in 'Wizard Rose' and 'Wizard Jade' cultivars. Coleus seedlings in the growing pots were collected and measured on each component: radicle, hypocotyl and leaves, the results obtained representing the average of each measured seedling component. Figures 4-5 show the influence of Pb(II) stress on radicle, hypocotyl and leaf development in 'Wizard Rose' and 'Wizard Jade'.

Analysing each component separately, it can be seen that the presence of Pb(II) affected the radicle most in both varieties. In 'Wizard Rose' the radicle length starts at 3.20 cm at 25 mg/kg Pb(II) and reaches a length of 1.72 cm at 300 mg/kg Pb(II) (Figure 2).

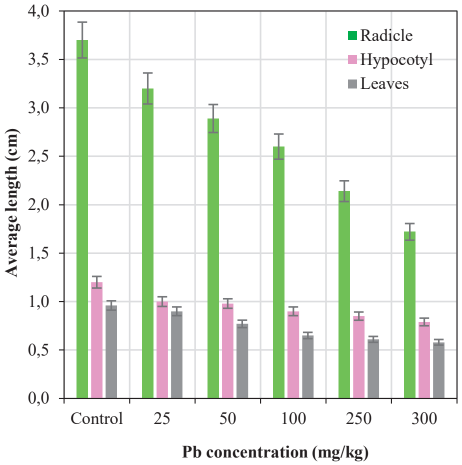

Figure 2. Influence of Pb(II) stress on the development of seedlings 'Wizard Rose' cultivar

In the case of Coleus 'Wizard Jade' a radicle length of 2.80 cm was recorded at 25 mg/kg Pb(II) concentration and reduced to 1.43 cm at 300 mg/kg Pb(II) concentration (Figure 3).

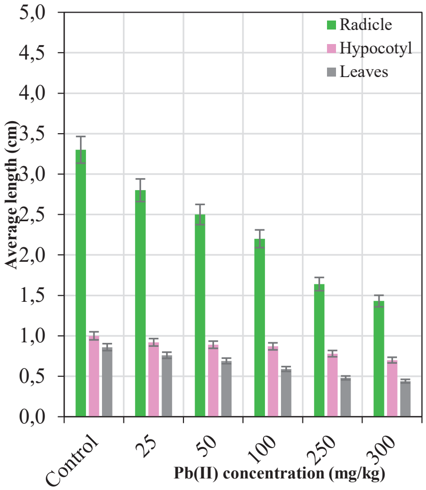

Figure 3. Influence of Pb(II) stress on the development of seedlings 'Wizard Jade' cultivar (cm)

Comparing with the control sample where the radicle length is 3.7 cm in 'Wizard Rose' and 3.3 cm in 'Wizard Jade', in both varieties the increase in Pb(II) concentration causes a reduction in radicle growth (radicle length decreases by 53.51% in 'Wizard Rose' and 56.66% in 'Wizard Jade'). The same trend of decreasing values due to increasing Pb(II) concentration is observed for hypocotyl length and leaf length. It can be seen that at the concentration of 300 mg/kg Pb(II), hypocotyl length decreases compared to the control sample with 34.17% in 'Wizard Rose' and 30.00% in 'Wizard Jade'. In terms of leaf size, leaf length decreases compared to the control sample by 39.58% in 'Wizard Rose' and 48.84% in 'Wizard Jade'

Degree of toxicity of Pb(II) to the radicle, hypocotyl and leaves of 'Wizard Rose' and 'Wizard Jade'.

Another indicator reflecting the inhibitory effect of lead for the two coleus varieties is the toxicity index (%). Thus, in Figures 4-5, the degree of toxicity of Pb(II) to the radicle, hypocotyl and leaves of 'Wizard Rose' and 'Wizard Jade' is represented. In both cultivars, in the case of Pb(II) contamination of samples (Figures 4-5), the degree of toxicity increases with the concentration of metal in solution and follows the order radicle>leaf>hypocotyl. In 'Wizard Rose', the toxicity degree values for radicle, hypocotyl and leaves show higher values with increasing Pb(II) concentration in the substrate. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 53.51% in the radicle, 34.17% in the hypocotyl and 39.58% in the leaves.

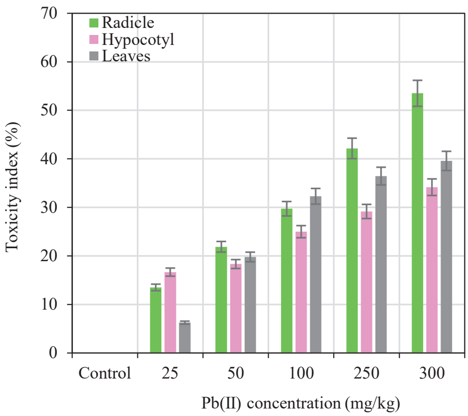

Figure 4. Influence of Pb(II) stress on the toxicity index 'Wizard Rose' cultivar (%)

In 'Wizard Rose', the toxicity degree values for radicle, hypocotyl and leaves show higher values with increasing Pb(II) concentration in the substrate. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 53.51% in the radicle, 34.17% in the hypocotyl and 39.58% in the leaves. The toxic effect of lead induced in 'Wizard Jade' not only a much lower germination percentage but also much higher toxicity index values compared to 'Wizard Rose' cultivar. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 56.67% in the radicle, 30.00% in the hypocotyl and 48.84% in the leaves.

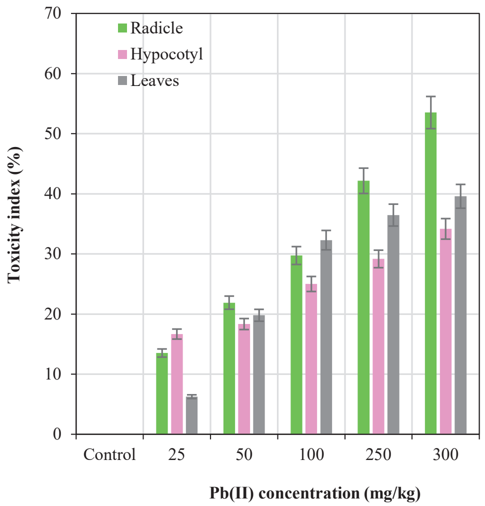

Figure 5. Influence of Pb(II) stress on the toxicity index 'Wizard Jade' cultivar (%)

These results are in agreement with the results of other studies confirming that the presence of Pb(II) in concentrations of 25-300 mg/L decreases radicle length: in *Lavandula angustifolia* L. (Hagiu Zalenschi et al., 2022), in *Brassica napus* L. (Rosca et al., 2021), in *Miscanthus* (Hsu et al., 1992).

Degree of tolerance of Coleus 'Wizard Rose' and Coleus 'Wizard Jade' varieties to Pb(II) stress

Figures 6-7 show the degree of tolerance of 'Wizard Rose' and 'Wizard Jade' to Pb(II) stress for radicle, hypocotyl and leaves. The tolerance of 'Wizard Rose' and 'Wizard Jade' to Pb(II) stress for radicle, hypocotyl and leaves is shown in the two graphs. Also for this indicator, the radicle is the most affected component of the seedling compared to the other components. With increasing Pb(II) concentration from 50 mg/kg to 300 mg/kg, the order of tolerance of seedling components is hypocotyl>leaf>root (Figures 6-7).

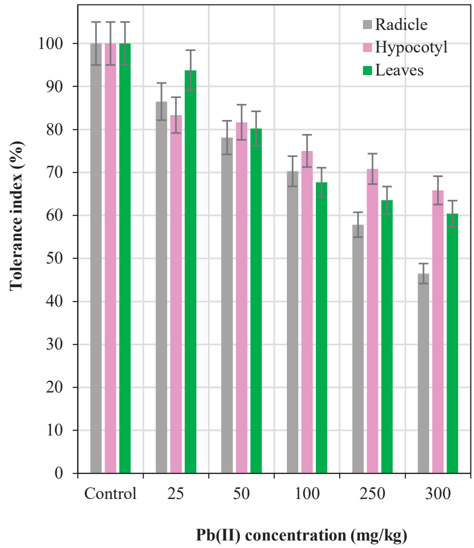

Figure 6. Influence of Pb(II) stress on the tolerance index 'Wizard Rose' cultivar (%)

At the radicle level, with increasing Pb(II) concentration from 25 mg/kg to 300 mg/kg the tolerance index decreases, with values ranging from 86.49% to 46.49 in Coleus 'Wizard Rose' and from 84.85% to 43.33 in Coleus 'Wizard Jade'.

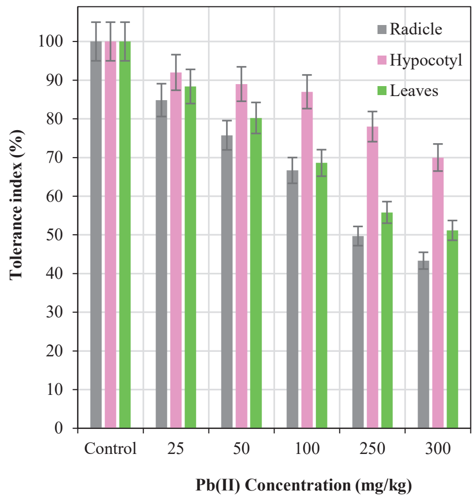

Figure 7. Influence of Pb(II) stress on the tolerance index 'Wizard Jade' cultivar (%)

In hypocotyl and leaves the decrease of the tolerance index is evident with the increase of Pb(II) concentration in the substrate, with very low values at the concentration of 300 mg/kg

(65.83% in hypocotyl and 60.42% in leaves in 'Wizard Rose' and 70% in hypocotyl and 51.16% in leaves in 'Wizard Jade').

Similar results were obtained by the study of the germination characteristics of *Lavandula angustifolia* under Pb(II) stress (Hagiu Zalenschi et al., 2022).

Vigour index of 'Wizard Rose' and 'Wizard Jade' varieties to Pb(II) stress.

Concerning the vigour of the seedlings of the two coleus varieties, the results show in all Pb(II)-contaminated variants of the two varieties a decrease in vigour index with increasing concentration. The vigour index values ranged from 5.00 at 25 mg/kg Pb(II) to 3.22 mg/kg at 300 mg/kg Pb(II) in 'Wizard Rose' (Figure 8).

In 'Wizard Jade' the seedling vigour index decreased with increasing concentration from 25 to 300 mg/kg, from 4.34 to 2.89 (Figure 8).

Our results confirm the results of other studies that lead accumulation in the soil and the plants inhibits germination of seeds and retards growth of seedlings, decreases germination percent, germination index, root/shoot length and tolerance index (Nas & Ali, 2018; Qu et al., 2021).

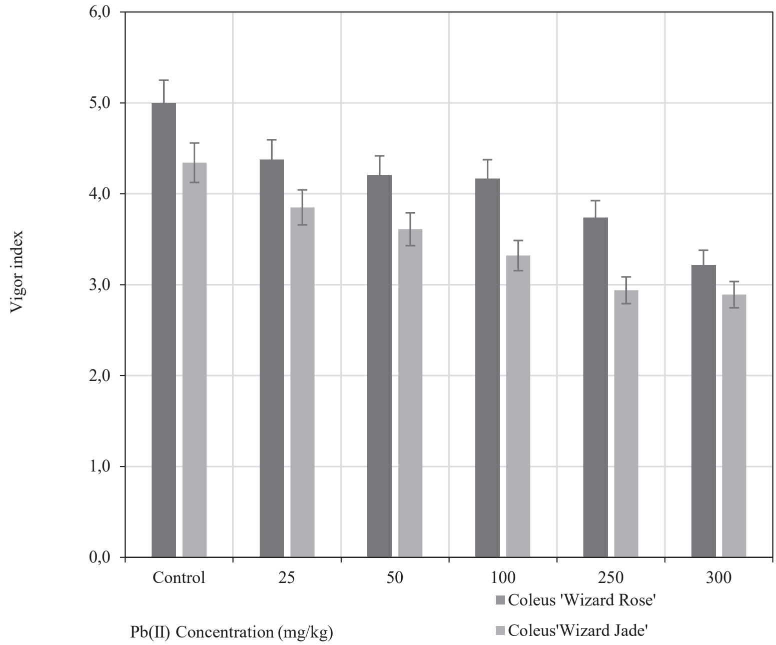

Figure 8. Influence of Pb(II) stress on the vigor index 'Wizard Rose' and 'Wizard Jade' cultivars

CONCLUSIONS

The germination percentage of seeds of the two coleus varieties under stress conditions caused by the presence of Pb(II) in concentrations of 300 mg/kg shows a significant decrease of 20% in the case of 'Wizard Rose' and 50% in the case of 'Wizard Jade'.

The seedling sprouting dynamics of the two coleus varieties started 7 days after sowing and lasted for six days in the control and eight days in the other experimental variants, suggesting that seedling sprouting was not significantly influenced by lead toxicity.

The dynamics of germination rate in the two coleus varieties indicate some variations determined, in particular, by the concentration of lead in the substrate, both varieties in the lead-contaminated variants had a lower germination rate than the control.

The velocity of germination rate was unfavourably influenced by the increase of Pb(II) concentration in the substrate with different values between varieties and variants and during the sprouting period.

The toxicity index for the two varieties follows the order: 'Wizard Jade'> 'Wizard Rose', increases with the metal concentration in the substrate and at the seedling level for both varieties the order is radicle > leaves > hypocotyl.

The tolerance index of the two varieties decreases with increasing metal concentration in the substrate and at the seedling level for both varieties the order is hypocotyl>leaf>radicle. The following tolerance scale was obtained for the varieties studied: 'Wizard Rose'> 'Wizard Jade'.

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THE INFLUENCE OF SOME GROWTH REGULATORS ON THE GERMINABILITY AND DEVELOPMENT AT *ALBIZIA JULIBRISSIN* DURAZZ.

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Abstract

Among the multitude of dendrological species with high decorative value, Albizia genus is of particular importance in the landscape design especially in the conditions of our country which are generally favorable and very favorable for the growth of these species. The purpose of the present work is to highlight the ornamental potential of the best known species of Albizia genus. Albizia julibrissin Durazz. *seeds were used for the experiment. Propagation of this species can be done quite easily by seeds, provided that the pods are harvested in October and the sowing is done in the spring (Sandu, 2009). Observations were made regarding the evolution in different periods of the vegetation, aiming the development regarding the germinability, growth and development of seedlings under the action of some rooting regulators, namely Radi-Stim Nr. 2, Asfac-4 25 ppm and Asfac-4 20 ppm. The variants treated with these regulators had a particular importance on seed germination, in terms of a deeper rooting, resulting in much more vigorous plants.*

Key words*: seedlings, variants, growth regulators, Albizia, germinability.*

INTRODUCTION

One of the basic components of the green spaces that ensure the aesthetic appearance of the localities is the landscaping. Among the multitude of dendrological species of ornamental trees, the species of the genus *Albizia* are of particular importance in the arrangement of green spaces and the conditions in our country are generally favourable and very favourable for this specie.

Albizia julibrissin Durazz. is a tree that reaches 12 m heights. It is characterized by a showy crown, slightly branched, with divergent branches (Sandu, 2009). It has bipinnate leaves with up to 12 leaflets. The flowers are pink, grouped in spherical heads (Mohan et al., 2010) and forms 12-13 cm long flat pods, light brown in colour (Iliescu, 2002).

The need to diversify the assortment is imposed as a priority considering the diversity of the biological material and above all the achievements reached worldwide.

Plant propagation sector registered technical innovations (automatic mist propagation unit, basal heating) that provide optimal environmental condition to improve rooting efficiency (Ferrante et al., 2013).

Despite of the optimal environment cuttings are exposed to various stress factors during rooting due to the lack of roots and root functions.

Rooting and growth regulators are generally used in plant production to improve mineral intake efficiency, enhance plant resistance and tolerance to abiotic and biotic stress and also stimulate some physiological processes related to development (Cojocariu et al., 2019; Filiti et al., 1986; Petrozza et al., 2013).

This work complements the researches in the field, as an effective and well-documented material because it tracks seed germination, the rooting percentage and the development of *Albizia julibrissin* plants under the action of two growth regulators. Propagation of this species can be done quite easily by seeds. Pods are harvested in october and the sowing of the seeds is done in the spring. (Szabóet al., 2013; Zaharia et al., 2003).

MATERIALS AND METHODS

The aim of our research was testing the effect of growth regulators on *Albizia julibrissin* Durazz. seed germination process.

In our study, namely Radi-Stim powder, Asfac-4 25 ppm and Asfac-4 20 ppm regulators were tested in dendrological crops such as *Albizia* to speed up growth reducing the nursery.

The experimental design is the following:

- Seeds treated with **Radi-Stim powder**: treated variant (Vt) and a control variant (Vc);
- Seeds treated with **Asfac-4 variant**: treated variants (V1, V2) and a control variant (Vc).

1. Radi-Stim powder variant

For this variant seeds were powdered with Radi-Stim powder and then sown on 28.02.2022.

100 seeds were used, placed in alveolar pallets divided as follows:

- 50 seeds were treated with Radi-Stim powder (treated variant).
-50 untreated seeds (control) on a substrate with pH between 7-7.5.

By the time the seed germinate, watering was done at least four times a week.

2. Asfac-4 variant

For Asfac-4 variant seeds were sown on 14.04.2022.

- 50 seeds-treated with Asfac-4, 20 ppm, immersed for 4 hours in the solution;
- 50 untreated seeds (control) watered for one hour;
- 50 seeds treated with Asfac-4, 25 ppm, immersed for 4 hours in the solution.

Sowing was carried out after immersion in the solution, in the alveolar pallet in a substrate with a pH-u1 of 6.5-7.

Phytosanitary treatments

Due to the different conditions during the sowing period, the experimental variants required different treatments with phytosanitary substances:

- for **Radi-Stim** variants were performed only 3 treatments for *Trialeurodes vaporariorum*, using Actara 25 WG 0.02%.
- for **Asfac-4** variants were required treatments for both *Trialeurodes vaporariorum* using Actara 25 WG 0.02% (3 treatments), and against *Pythium debaryanum* using Previcur 607 SL 0.15% (3 treatments).

Observations regarding the emergence and growth of *Albizia julibrissin* Durazz seedlings were carried out daily, the measurements being recorded weekly.

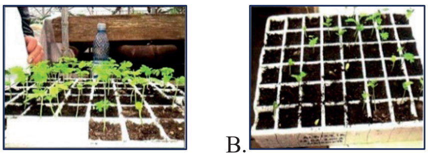
Data statistical processing was carried out based on variance analysis (limit differences, DL test) (Săulescu N.A., Săulescu N.N., 1967).

RESULTS AND DISCUSSIONS

Results for Radi-Stim variants

Seed germination took place on 07.03.2022, the percentage of germination being higher in the case of the treated variant than in the control one. Until the first measurement, the sprouted plants evolved in growth, some sprouted on 12.03.2022, the percentage of sprouting being 65% for the treated variant (Figure 1A) and 35% for the control (Figure 1B).

Table 1 presents plants growth between 13.03.2022 until 03.04.2022 and it can be observed the very significant growth of treated variant compared to the control.



A. B.

Figure 1.1st stage of plant growth, Radi-Stim variant:
A - Treated variant B - Control (untreated)

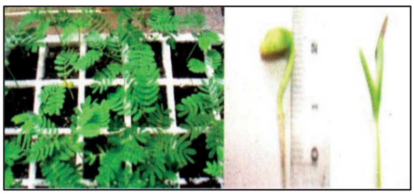


Figure 2. Cotyledon leaves stage

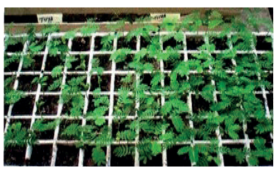


Figure 3. Experimental variants - 3rd stage of vegetation

Albizia plants have developed according to their stage, a part of them show the second layer of leaves, and the plants that have just emerged have obvious cotyledonal leaves (Figure 2).

As the plants grew, the cotyledon leaves began to disappear, entering the 3rd stage of vegetation (Figure 3). The emergence percentage was 79%.

Table 1. Results on plants growth, Radi-Stim variant at different date observation, data for year 2022

Variant	Observation time/ plants growth (cm)	
	13.03.2022	
	Average	DL 5% = 0.71 DL 1% = 1.12 DL 0.1%= 1.78
Vt	2.83^{***}	
Vc	0.63	
Variant	20.03.2022	
	Average	DL 5% = 0.84 DL 1% = 1.21 DL 0.1% = 1.87
Vt	3.00^{***}	
Vc	0.91	
Variant	27.03.2022	
	Average	DL 5% = 0,72 DL 1% = 1.00 DL 0.1% = 1.63
Vt	3.22^{***}	
Vc	1.11	
Variant	03.04.2022	
	Average	DL 5% = 0.61 DL 1% = 1.05 DL 0.1% = 1.57
Vt	3.65^{***}	
Vc	2.07	

Vt-Variant treated with Radi-Stim powder;
Vc- control untreated.

On 07.04. and 08.04 2022 *Albizia* plants were transplanted.

On 04.07.2022, at the treated variant followed a second transplantation because the germination percentage was 100%. The plants were vigorous, different in color and with a very well defined root system.

On 04/08/2022, the control variant was also transplanted, germination percentage being of 85%. The plants were smaller, less vigorous, differently colored and the root system well developed. The same type of substrate with pH 7.5 was used as in case of sowing. After transplantation plants were watered. The growth of the plants after transplanting recorded the average values registered in Table 2 and Figure 4.

Figure 4. *Albizia julibrissin* first transplantation

From 16.04.2022 the plants went through a period of cold so that the growths were no longer so obvious but the low temperature interval served as tempering (Table 2).

After the second transplantation (Figure 5) which is stimulated with Radi-Stim, the plants grow, the stem is obvious, starting to lignify, they are very tall.

Due to the fact that they have very fast growth and a much branched root system, plants require a new transplantation.

Figure 5. Plants second transplantation

Table 2. Results on plants growth after transplantation, Radi-Stim variant at different date observation, data for year 2022

Variant	Observation time/plants growth (cm)	
	16.04.2022	
	Average	DL 5% = 0.61 DL 1% = 0.93 DL 0.1% = 1.72
Vt	1.35^{*}	
Vc	0.83	
Variant	05.05.2022	
	Average	DL 5% = 0.54 DL 1% = 0.81 DL 0.1% = 1.67
Vt	1.73^{*}	
Vc	1.06	
Variant	16.05.2022	
	Average	DL 5% = 0.87 DL 1% = 1.35 DL 0.1% = 2.54
Vt	2.75^{*}	
Vc	1.35	
Variant	26.05.2022	
	Average	DL 5% = 0.76 DL 1% = 1.14 DL 0.1% = 2.11
Vt	3.07^{*}	
Vc	2.06	
Variant	06.06.2022	
	Average	DL 5% = 0.74 DL 1% = 1.15 DL 0.1% = 2.19
Vt	3.5^{*}	
Vc	2.71	
Variant	26.06.2022	
	Average	DL 5% = 0.83 DL 1% = 1.37 DL 0.1% =2.54
Vt	3.73	
Vc	3.01	
Variant	26.07.2022	
	Average	DL 5% = 1.04 DL 1% = 1.66 DL 0.1% = 3.11
Vt	4.01	
Vc	3.37	
Variant	16.08.2022	
	Average	DL 5% = 1.06 DL 1% = 1.65 DL 0.1% = 3.14
Vt	4.39	
Vc	3.75	
Variant	02.09.2022	
	Average	DL 5% = 0.93 DL 1% = 1.57 DL 0.1% = 2.98
Vt	4.93	
Vc	3.99	
Variant	21.09.2022	
	Average	DL 5% = 0.82; DL 1% = 1.35; DL 0.1% = 2.47
Vt	5.52^{*}	
Vc	4.64	
Variant	01.10.2022	
	Average	DL 5% = 0.97; DL 1% = 1.54;
Vt	5.88^{*}	

Vc	4.88	DL 0.1% = 2.76
Variant	21.10.2022	
	Average	DL 5% = 0.91 DL 1% = 1.58 DL 0.1% = 2.83
Vt	6.06^{*}	
Vc	4.96	

Vt - variant treated with Radi-Stim powder;
Vc - control untreated.

At the measurements from 06.06.2022, the plants registered significant growth, watering was reduced and treatments for *Trialeurodes vaporariorum* were applied.

In the following interval, the plants grew and developed according to the phenophase of vegetation. Until the end of the vegetation period, some specimens reached a height of 12-13 cm high and others remained in the same vegetation stage due to the temperature differences between night and day.

The losses during the winter were around 20% in Radi-Stim variant (Table 3, Figure 6).

Table 3. Results on plants growth Radi-Stim variant, data for year 2023

Variant	Observation time/ plants growth (cm)	
	03.04.2023	
	Average	DL 5% = 0.88 DL 1% = 1.26 DL 0.1% = 2.35
Vt	6.37^{*}	
Vc	5.40	
Variant	18.04.2023	
	Average	DL 5% = 0.81 DL 1% = 1.45 DL 0.1% = 2.68
Vt	6.84^{*}	
Vc	5.80	

Vt - variant treated with Radi-Stim powder;
Vc - control untreated.

Figure 6. Plants growth, Radi-Stim variant, second year of vegetation

Results for Asfac-4 variants

On 29.04.2022, the first plants from the alveolar pallet began to sprout, the seeds being treated with Asfac-4 in 2 concentration variants (20 and 25 ppm).

Transplanted plants continued their growth and, in some cases, due to the lower temperatures in the greenhouse at night, part of the leaves began to dry (Figure 7 and 8). At this point, treatments for *Trialeurodes* were carried out. The observations regarding plants growth from May to June are recorded in Table 4.

V1 - 20 ppm V2 - 25 ppm

Figure 7. Plants emergence, Asfac-4 variant

Figure 8. Plants growth -control -untreated

Table 4. Results on plants growth at the variant treated withAsfac-4, data for 2022

Variant	Observation time/ plants growth(cm)	
	5.05.2022	
	Average	
V1	0.22	DL 5% = 0.27 DL 1% = 0.39 DL 0.1% = 0.51
V2	0.45^{*}	
Vc	1.15	
Variant	16.05.2022	
	Average	DL 5% = 0.43 DL 1% = 0.58 DL 0.1% = 0.95
V1	1.05^{*}	
V2	0.96^{*}	
Vc	0.59	
Variant	26.05.2022	
	Average	DL 5% = 0.51 DL 1% = 0.74 DL 0.1% = 1.23
V1	1.47^{*}	
V2	1.40^{*}	
Vc	0.82	
Variant	26.06.2022	
	Average	DL 5% = 0.36 DL 1% = 0.48 DL 0.1% = 0.74
V1	2.67^{***}	
V2	2.34^{**}	
Vc	1.72	

V1- variant treated with Asfac-4 20 ppm;
V2- variant treated withAsfac-4 25 ppm;
Vc- control untreated.

In the control variant, 6 plants were removed because they withered, being necessary to eliminate them from experiment and 4 were eliminated from the treated variant.

On 23.05.2022 a treatment was carried out for *Pythium*.

Also in variant treated with Asfac-4 the plants grew (Figure 9), those that sprouted entered 2nd stage of development, the color of the leaves is different from light green at the time of growth to a dark green with obviously colored edges and veins in carmine red. The emergence percentage oscillates reaching at the moment 80% in the variant treated with Asfac-4 25 ppm, 65% in the variant treated with Asfac-4 20 ppm and only 40% in the control variant.

Figure 9. Sown variant treated with Asfac-4, 2nd stage of vegetation

From 26.05. the plants developed further, in those where Asfac-4 was used, some entered the 3rd stage of vegetation. At this stage, leaves on the edge and the main vein are colored carmine red which is characteristic of the specie.
The treatment for *Pythium* did not give much results because they were lost around 15 seedlings out of their total, and the ones that are just emerging are also affected (Figure 10).

Figure 10. Plants treated with Asfac-4, 3rd stage of vegetation

From 26.06.2022 the plants grew, and those from the transplanted version were transplanted again on 12.07. in much larger pots because the root system could no longer develop normally, needing more space. The substrate has a pH between 6.5-7 (Figure 11).
It followed a second transplanting on 12.07.2022 at treated version and on 13.07.2022 at the control version.
On 27.07.2022, for all variants, were carried out phytosanitary treatments, removal of weeds, dry leaves and soil loosening.

Figure 11. Plants after transplantation - Asfac-4 variant

From 16.08.2022 the plants registered new growth (Table 5), in both variants, treated with Asfac-4, 20 ppm and 25 ppm. Then, plants were taken out of the greenhouse to adjust to the temperature fluctuations during the summer.

Table 5. Results on plants growth at the variant treated with Asfac-4, after transplantation, data for 2022

Variant	**Observation time/ plants growth(cm)**	
	26.07.2022	
	Average	
V1	0.35	DL 5% = 0.25
V2	0.30	DL 1% = 0.57
Vc	0.25	DL 0.1% = 2.62
Variant	**16.08.2022**	
	Average	
V1	0.81	DL 5% = 0.43
V2	1.30	DL 1% = 1.07
Vc	0.45	DL 0.1% = 3.24
Variant	**02.09.2022**	
	Average	
V1	1.35^{**}	DL 5% = 0.23
V2	1.05^{*}	DL 1% = 0.46
Vc	0.80	DL 0.1% = 1.31
Variant	**21.09.2022**	
	Average	
V1	2.23^{**}	DL 5% = 0.48
V2	1.57^{*}	DL 1% = 0.93
Vc	1.10	DL 0.1% = 2.87
Variant	**01.10.2022**	
	Average	
V1	2.40^{*}	DL 5% = 0.50
V2	1.90	DL 1% = 1.14
Vc	1.45	DL 0.1% = 3.49
Variant	**21.10.2022**	
	Average	
V1	2.85^{*}	DL 5% = 0.61
V2	2.05	DL 1% = 1.40
Vc	2.50	DL 0.1% = 4.57

V1-variant treated with Asfac-4 20 ppm;
V2- vatiant treated with Asfac-4 25 ppm;
Vc- control untreated.

Subjection to climatic factors is a very good thing because they have behaved very well, the root system is well developed, the color of the plants is different, oscillating from dark green to light green when another layer of leaves grows, having the edge leaves colored with carmine red.
Since 02.09.2022, the plants have been treated again for *Pythium* and care works consisting of weeding, breaking the crust, loosening the soil.
Growth is slower in the plants that have been transplanted twice, a sign that the seedlings are gradually preparing for winter, while in the part stimulated with Asfac-4 they continue to grow, taking the plants outside only on warmer days to avoid plant breakage caused by wind and rain.
Significant and distinct significant diferences can be seen in case of 20 ppm variant.

Since 21.09.2022 the plants have undergone some changes, the growth being very small.
Since 01.10.2022 there have been no further changes, the growth is no longer visible, growing only by 5 mm in some plants. Being a deciduous tree, it prepares to enter the period of vegetative rest, stopping all processes of growth and development.
The plants were brought indoors to prevent the wind from destroying the plants.
Throughout the resting period, the plants were watered, kept at the optimal temperature to avoid frost, as they are sensitive to low temperatures.
On 15.03.2023 the plants started to grow, a sign that they resumed their vegetative stage.
Losses over the winter were around 15% in the variant stimulated with Asfac-4.

Table 6. Results on plants growth at the variant treated with Asfac-4, year 2023

Variant	Observation time/ plants growth (cm)	
	03.04.2023	
	Average	
V1	2.92**	DL 5% = 0.33 DL 1% = 0.74 DL 0.1% = 2.37
V2	2.25*	
Vc	1.65	
Variant	18.04.2023	
	Average	
V1	3.70**	DL 5% = 0.65 DL 1% = 1.41 DL 0.1% = 4.58
V2	2.90*	
Vc	2.20	

V1- variant treated withAsfac-4 -20 ppm;
V2- variant treated with Asfac-4 25ppm;
Vc- control untreated.

Both variants, Radi-Stim and Asfac-4 resumed their vegetation cycle, the differences between them being a few days.
The plants started to grow even more and colored light green in the variant treated with Radi-Stim and dark green in the variant treated with Asfac-4.
The growth is more obvious in Radi-Stim variant than in the variant treated with Asfac-4.
Plants had increased resistance to diseases and pests, have a well-developed root system. The plants high reached up to 27 cm.
Since 03.04.2023 the plants have recovered, they have grown (Table 6), watering was done more and more and also care work has been carried out which consists of: removing dry plants, weeding the weeds, breaking the crust, filling the pots with soil, aerating the root system.

Discussions
Seed germination occurred 8 days after sowing, most of them from the variant treated with Radi-Stim powder. However, most of the seedlings emerged around 12.03.2022, the results being obviously favorable for the variant treated with Radi-Stim powder compared to the control version, namely: 67.5% for the treated variant, compared to 31.25% for the control, untreated.
During the observations, obvious differences were noted between the two variants regarding the speed and percentage of germination, the variant treated with Radi-Stim powder being superior to the control by 5-25%.
Treatments using rooting regulators favoured seed germination, plant emergence and positively influenced the germination percentage, duration and speed of emergence also in case of other reserches using perenials (Cojocariu, 2019; Ferrante, 2013).
Figure 12 shows the growth evolution from the moment the seed germinates until transplanting; the measurement values match the interval March 13 till April 3, 2022.
The plants were transplanted on 7 and 8 of April, 2022, for the variant treated with Radi-Stim powder, with a 100% planting success.
Figure 13 shows the evolution of plant growth from replanting until plants went dormant, the measured values corresponding to the interval April 16 - October 21, 2022.

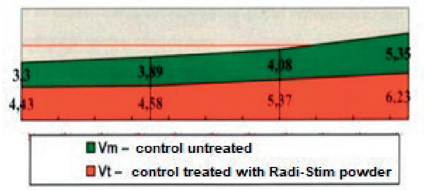


Figure 12. Growth evolution from the moment the treated seeds germinate until transplanting, Radi Stim variant

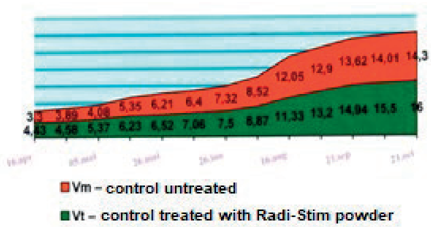


Figure 13. Evolution of plant growth from replanting until plants went dormant, Radi Stim variant

On 14.04.2022, the second experience was set, in order to compare the behavior with a germination stimulator from another category of active substances. For this, Asfac-4 was used, a growth stimulator with a phenoxyacetic nucleus substituted with sulfonamide groups, which is tested and approved on otherspecies and varieties (Petrozza, 2013; Szabó, 2013).

At this experiment (Asfac-4) the first seedlings emerged on 29.04.2022, but still with a delay of approx. 7 days compared to Radi Stim experiment.

Partial results so far highlight the fact that the sowing in the 3 variants was directly influenced by the environmental conditions but also by the lateness of the sowing time.

The observations made between 05.05 - 26.05 2022 highlighted the following aspects:

- germination was lower, varying between 50 and 78%, compared to the version treated with Radi-Stim where this percentage was 100%;
- the growth recorded in the sprouted seedlings had a lower rate, after approx. 3 weeks the plants having the average dimensions between 5 and 5.5 cm, while in the first experience, in the same time interval, the growths measured were, on average, 5.5-6.5 cm.

There where made observations of plants rooted after being treated with Asfac -4. Figure 14 highlights the growth evolution of these plants from germination until the time of transplanting, between 05.05. and 12.07.2022.

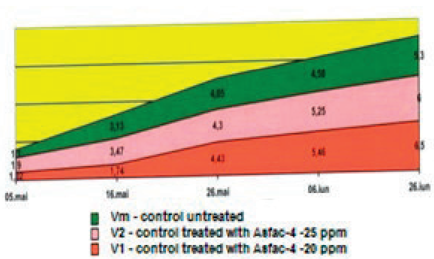


Figure 14. Plants growth evolution from germination until transplanting - Asfac-4 variant

Figure 15 shows the evolution of plant growth from transplanting until they went dormant. The measured values are included in the interval July 10 - October 21, 2022.

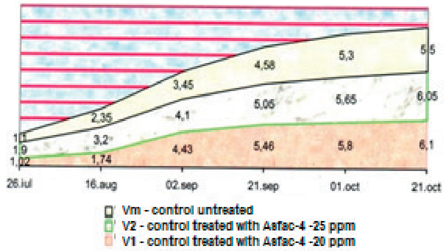


Figure 15. Plants growth evolution until dormancy, Asfac-4 variant

CONCLUSIONS

According to the observations made and the results on the evolution of growth stimulants used in *Albizia julibrissin*, it can be concluded that variants treated with growth regulators had a special importance on seed germination, formation of a deep root system and much more vigorous plants.

Seedling emergence occurred earlier (after approx. 8 days from sowing) in the case of the Radi-Stim powder treatment, the germination percentage varying between 67.5% and 100%.

Significant positive influence of the Radi-Stim product was noted only on the seeds germination percentage. Plants growth recorded during the observation interval 13 March 2022-18 April 2023 are based on the advantage that treated variants develop a more branched and healthier root system. The most obvious differences are noted at the level of the root system, where the plants from the variant treated with Radi-Stim showed obviously larger and richly branched roots.

In the case of the second variant, soaking the seeds in Asfac-4 solutions, it was highlighted that the germination was lower than in the case of seeds treatment with Radi-Stim, a fact that was also influenced by the less favourable environmental conditions during the experimental period.

From the analysis of the results regarding how the species reacts when a growth regulator is used, it appears that the best variant is the variant treated with Radi-Stim powder, and in the case of using the Asfac-4 product, the best variant is V1 - the variant treated with 20 ppm.

In case of Radi-Stim treatment variant, a better percentage of plant emergenceswere recorded, explained by the fact that the optimal sowing time was respected, compared to Asfac-4 treatment variant, where sowing was carried out at the beginning of summer.

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PAULOWNIA IMPERIALIS, PRINCESS PAVLOVA'S TREE: DEVELOPING A PLANTATION IN ROMANIA

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Abstract

Paulownia is the fastest-growing tree species in Romania compared to other dendrological species. The development of this species for commercial purposes can be achieved in 4-6 years if certain pedological, agrochemical, and agrotechnical factors are respected, along with specific maintenance work. This study analyzed two hybrid varieties: Paulownia 'Cotevisa 2' and Paulownia 'Superhibrid Z 07'. The establishment and maintenance work, as well as the growth evolution of these varieties, were monitored. The main objective is to obtain data that contribute to the success of Paulownia cultivation in Romania, due to the lack of information regarding the cultivation and maintenance of this plant.

Key words: *paulownia, timber, flowers*

INTRODUCTION

Paulownia cultivation in Romania is a growing agricultural practice, with increasing interest in recent years. *Paulownia* is a tree native to Asia, also known as the "imperial tree" or "porcelain tree". It is characterized by its rapid growth and adaptability to various soil and climatic conditions (Bernardis, 2011).

In Romania, *Paulownia* cultivation is promoted primarily for its valuable timber and its use in the furniture industry. *Paulownia* wood is appreciated for its unique properties, such as lightweightness, strength, and durability. It is also non-toxic and resistant to moisture, making it suitable for various applications. *Paulownia* cultivation in Romania can be carried out in different regions, as the tree adapts to different soil types and climatic conditions. However, attention should be given to soil preparation and proper care to achieve optimal results.
(Zaharia, 2003; Dumitraș, 2003).

Another interesting aspect of *Paulownia* cultivation in Romania is its potential for ecological purposes. Due to its ability to absorb large amounts of carbon dioxide from the atmosphere, *Paulownia* can contribute to reducing greenhouse gas emissions and improving air quality. In conclusion, *Paulownia* cultivation in Romania represents an interesting opportunity for farmers and investors, thanks to the fast growth of the trees, the value of the timber, and its ecological potential. However, further research and consultation with experts are important before initiating such cultivation, as its success depends on various factors, including proper crop management and identifying potential markets for the products obtained (Simion, 2009).

MATERIALS AND METHODS

The study included two hybrids of *Paulownia*: 'Superhibrid Z 07' and 'Cotevisa 2'.varieties within the "*Paulownia* Șipote" plantation. The plantation is located 55 km away from Iași, on a land in the Șipote commune, and is intended for the production of healthy trees that will become an important source of timber and, consequently, profit. The locality of Șipote in Iași county enjoys favorable climatic and soil conditions. In terms of climate, the area is characterized by a temperate-continental climate, with warm summers and cold winters. The average temperatures range from 0°C in January to 25°C in July, with moderate precipitation throughout the year.

The soils in Șipote are generally rich in nutrients and have good drainage. They are mainly brown-reddish soils and chernozems, which are highly fertile and suitable for agriculture. These types of soils provide good water and nutrient retention, which is beneficial for plant development.These climatic and soil conditions

in the locality of Șipote provide a conducive environment for various agricultural activities such as crop cultivation, animal husbandry, and fruit growing. Farmers in the area can benefit from these favorable conditions to obtain abundant and high-quality yields.
This plantation obtained the two analyzed varieties from the following sources:

1. ***Paulownia* Z 07** (**Superhybrid**) is a crossbreed of three species: *Paulownia tomentosa*, *Paulownia fortunei,* and *Paulownia kawakamii.* This hybrid of *Paulownia* is the most popular and preferred in Japan for its fast growth and high-quality wood. It is highly robust, with excellent growth rate, and can adapt to extreme temperature and soil conditions. This species of *Paulownia* is the most resistant to diseases, extreme heat (up to +40°C), drought, and cold (down to -33°C), making it the most frost-resistant among all species (Baier et al., 2021; Szabo et al., 2022).

It is ideal for plantations in Europe, where there are severe extreme temperatures and harsh frosts. In the second year after planting, it can reach a height of 6-8 meters, producing a tall, smooth trunk without knots and of superior quality. It does not have specific requirements for growth conditions. It can thrive in both light and heavy soils, but it grows best in moderately moist, permeable clay soils that are sufficiently fertile. Mature plants, due to their deep root system, are drought-resistant, but in the first year, periodic and abundant watering is necessary.

2. **The 'Cotevisa 2'** hybrid was cultivated in 2003 as a non-invasive hybrid of *Paulownia elongata* and *Paulownia fortunei.* After reaching the required quality, these individuals were reproduced through in vitro vegetative propagation.. *Paulownia* 'Cotevisa 2' was cultivated for semi-arid climatic conditions but is highly adaptable to local climatic conditions. This clone tolerates a temperature range from -25 to +40°C (Lachowicz et al., 2022).

The bifactorial experiments were organized in randomized blocks with three replications (five plants per replication):

- Factor A - genotype, with two variables: a1 - 'Superhibrid Z 07'; a2 - 'Cotevisa 2';
- Factor B - irrigation, with two variables: b1 - non-irrigated; b2 - irrigated. The combination of these factors resulted in four experimental variants: V_1 - 'Superhibrid Z 07' non-irrigated; V_2 - 'Superhibrid Z 07' irrigated; V_3 - 'Cotevisa 2' non-irrigated; V_4 - 'Cotevisa 2' irrigated. During the mentioned period, observations were made for the following parameters: height growth and stem diameter, based on hybrids and water administration. The statistical analysis of the data was performed using the analysis of variance method.

These varieties are often promoted for forestry and agricultural plantations, having several characteristics that make them attractive to farmers or investors in horticulture and forestry. Here are some features and information about them:
a. **Fast growth**: *Paulownia* 'Cotevisa 2' is known for its rapid growth rate, reaching commercial size within a relatively short period, usually between 3 and 4 years, making it attractive for timber production. In contrast, 'Superhibrid Z 07' has a slower growth rate, reaching maturity approximately two years later compared to 'Cotevisa 2'.
b. **Wood quality**: *Paulownia* wood is lightweight yet strong, with low density and good resistance to decay. It is often used in the manufacturing of furniture, panels, musical instruments, and even construction.
c. **Adaptability**: Both varieties are adapted to grow in a variety of climatic and soil conditions, although they prefer locations with sun exposure and well-drained soils.
d. **Disease resistance**: These varieties have been selected for their resistance to diseases and pests, reducing the need for chemical treatments and maintenance.
These determinations will be carried out during the period 2021-2025, and assessments have been made regarding tree growth rate, maintenance work, and agrochemical or insect-pest treatments.
In the second year, a technical cut was applied to all experimental variants, which involved shortening the main stem to 5 cm above ground level. Water administration in the irrigated variants was carried out at intervals of 6-7 days, with quantities of 10-15 liters per plant. Biometric determinations were conducted

throughout the vegetation period, focusing on plant height and stem diameter. The stem diameter was measured at a height of 1 m above ground level. All other maintenance work was performed in all experimental variants.

Statistical data processing was carried out based on variance analysis (limit significance differences, LSD test) (Săulescu et al., 1967).

RESULTS AND DISCUSSIONS

In our measurements, we used 3 trees of each species as control, which means that these trees did not receive proper care compared to the others, but they received the necessary minimum to survive.

It is important to note that these growths are correlated with proper care, including regular irrigation, fertilizer distribution, and soil mulching.

The difference in development between the control trees and the observed ones is significant, so planting them without a proper care plan is not recommended.

Agrotehnical Operations

In a *Paulownia* plantation, there are several agricultural operations necessary to ensure healthy growth and optimal production. These operations include:

1. **Soil preparation**: Before planting, the soil needs to be properly prepared. This may involve tasks such as scarification, plowing, tilling, or leveling the land, removing weeds, and improving soil fertility by applying organic or mineral fertilizers.
2. **Planting seedlings**: *Paulownia* seedlings are usually planted between April and November, depending on local climatic conditions. The seedlings should be healthy, with well developed roots, and should be spaced appropriately based on the desired plantation density.
3. **Irrigation**: In the early stages of development, *Paulownia* seedlings require regular watering to ensure healthy root and stem growth. Irrigation should be sufficient to keep the soil moist but not excessively wet to avoid waterlogging.
4. **Weed control**: Weeds can negatively affect the growth and development of *Paulownia* seedlings by competing for available resources. Soil milling or manual weed removal should be carried out to keep the plantation clean and minimize excessive competition.
5. **Fertilization**: Depending on soil analysis and specific plant requirements, organic or mineral fertilizers can be applied to provide adequate nutrition for *Paulownia* seedlings. This can be done through direct soil application or fertigation.
6. **Pest protection**: *Paulownia* can be affected by various pests, such as slugs, the hairy caterpillar (*Hyphatria cunea*), and the cereal bug (*Eurygaster integriceps*). It is important to monitor the plantation and take preventive or curative measures, such as insecticide treatments, to protect the trees from such issues. (Zaharia & Dumitraş, 2003).
7. **Technical cut**: Technical cut is an important operation for shaping and controlling the development of *Paulownia* trees. Selective pruning of branches and stems can achieve proper form and optimal timber production.

Paulownia cultivation can thrive in a variety of soil types, but there are certain soil characteristics that can favor healthy growth and optimal production. Here are some important considerations regarding soil for *Paulownia* cultivation:

a. **Good drainage**: The soil should have good drainage to avoid excessive water accumulation around the roots. Heavy clay soils or poorly drained soils can cause root problems and negatively impact the growth and development of *Paulownia* trees.

b. **Neutral to slightly acid pH**: The ideal soil for *Paulownia* has a pH between 6 and 8. Soils that are too acidic or too alkaline can affect nutrient absorption and negatively influence the health and growth of the trees.

c. **Moderate fertility**: The soil should be moderately fertile, with adequate nutrient content necessary for healthy growth. Soil analysis is recommended to determine nutrient levels and adjust fertilization accordingly.

d. **Good structure**: The soil should have a good structure that allows roots to develop and spread properly. Heavy clay soils can be improved by adding organic matter to enhance soil structure and drainage. It is important to conduct a soil evaluation before planting and consider the specific needs of *Paulownia* cultivation.

Consulting a soil specialist or agronomist can be helpful in obtaining specific soil advice and taking appropriate measures to improve soil quality if necessary.
The proper planting of *Paulownia* seedlings is a crucial step in developing a healthy and productive crop. Here are some important considerations regarding the planting of *Paulownia* seedlings:

a. **Timing**: *Paulownia* seedlings are usually planted between April and November, depending on local climatic conditions. It is important to choose a time when the risk of frost or extreme temperatures is minimal. (Poșta et al., 2022).

b. **Soil preparation**: The soil should be properly prepared before planting. This may involve tasks such as plowing, tilling, or leveling the land, removing weeds, and improving soil fertility by applying organic or mineral fertilizers. Soil preparation is visible in Figure 1.

Figure 1. Soil preparation

c. **Proper spacing**: It is important to maintain appropriate spacing between seedlings during planting. This may vary depending on the desired plantation density and the species of *Paulownia* being cultivated. Typically, a distance of approximately 3-4 meters between seedlings is recommended.

d. **Planting holes**: Planting holes should be deep and wide enough to allow proper root development. Adding compost or other organic materials to the planting hole is recommended to improve soil fertility and provide additional nutrients to the seedlings.

e. **Careful handling of roots**: Before planting, ensure that the roots of the *Paulownia* seedlings are healthy and well-developed. Handle the roots with care to avoid damage and plant the seedlings in the prepared holes immediately after removing them from the transport container.

f**. Initial watering and care**: After planting, ensure that the *Paulownia* seedlings are well-watered to provide sufficient moisture for them to establish in the soil. Regularly monitor soil moisture and ensure that it remains moist but not saturated with water. Also, protect the seedlings from herbivores and weeds by implementing appropriate protective measures.
It is important to follow specific instructions from the *Paulownia* seedling producer and consider local conditions and specific requirements of the cultivated species. Consulting a *Paulownia* crop specialist or agronomist can be helpful in obtaining advice and specific information for successful *Paulownia* seedling planting.
Proper watering of *Paulownia* seedlings is essential to ensure healthy growth and optimal root development. Here are some important considerations regarding watering *Paulownia* seedlings:

a. **Water quantity**: *Paulownia* seedlings require an adequate amount of water to develop properly. Generally, providing approximately 10-15 liters of water per week for each seedling is recommended. However, the exact amount of water may vary depending
on local climatic conditions, soil, and the stage of seedling development. (Baier et al., 2021).

b. **Watering frequency**: It is important to keep the soil around the *Paulownia* seedlings moist but not excessively wet. Regular and consistent watering is usually more effective than heavy watering once a week. Monitor soil moisture and ensure that it remains moist but not waterlogged.

c. **Timing of watering**: In general, it is recommended to water *Paulownia* seedlings early in the morning or late in the evening to minimize water evaporation and allow roots to efficiently absorb water. Avoid watering during the day when temperatures are high and evaporation is greater.

d. **Watering techniques**: There are several watering methods that can be used for *Paulownia* seedlings, such as drip irrigation, sprinkler irrigation, or manual watering with a hose or bucket. Choose the method that works best for you and ensure that water reaches the roots directly (Morenoa et al., 2017).
It is important that water reaches the plants, regardless of the watering method used (Figure 2).

Figure 2. Rustical manual irrigation, with the farm's equipment

Adaptation to local conditions
Consider the specific climate and soil conditions in your area and adjust the watering schedule accordingly. Sandy soils may require more frequent watering, while clay soils may retain water for longer periods. It is important to constantly monitor the condition of the *Paulownia* seedlings and adjust the watering schedule based on their needs. Ensure that the soil remains moist but not excessively wet to avoid root problems and promote healthy seedling growth.

The fertilizer used for *Paulownia* seedlings can play an important role in ensuring adequate nutrition and healthy growth. Here are some important considerations regarding fertilizer for *Paulownia* seedlings (Beckjord et al., 1983; Morenoa et al., 2017):
a. **Soil analysis**: Before applying fertilizer, it is recommended to conduct a soil analysis to determine the level of available nutrients and identify any deficiencies. This can help you choose the appropriate fertilizer and adjust the dosage based on the specific needs of the soil and *Paulownia* seedlings.
b. **Organic fertilizers**: The use of organic fertilizers can be beneficial for *Paulownia* seedlings as they provide essential nutrients and improve long-term soil fertility. Examples of organic fertilizers can include compost, composted manure, or other organic materials.
c. **Mineral fertilizers**: Mineral fertilizers can be used to provide essential nutrients in an easily assimilable form for *Paulownia* seedlings. These may include fertilizers with nitrogen, phosphorus, potassium, and other elements necessary for healthy growth. The dosage and composition of mineral fertilizers may vary based on the specific needs of the soil and manufacturer recommendations.
d. **Application methods**: Fertilizer can be applied in various ways, such as direct soil application around the roots, application in the planting hole, or through fertigation (applying fertilizer through the irrigation system) (Wu et al., 2022). Ensure that you follow the manufacturer's instructions and apply the fertilizer in appropriate doses to avoid over or under-dosage.
e. **Monitoring and adjustment**: It is important to monitor the condition of the *Paulownia* seedlings and observe any signs of nutrient deficiencies or fertilizer excess. Adjust the dosage and frequency of fertilizer application based on the specific needs of the seedlings and their response to the fertilizer. Consulting a plant nutrition specialist or agronomist can be helpful in obtaining specific advice and appropriate recommendations for *Paulownia* seedling fertilization, considering local conditions and specific crop requirements.

Weed control in *Paulownia* seedlings is essential to ensure healthy growth and optimal development. Weeds can compete with the seedlings for available resources such as water, light, and nutrients, and can negatively impact their growth and development. Here are some important considerations regarding weed control in *Paulownia* seedlings:

a. **Manual removal**: An effective and environmentally friendly method of weed control is manual removal. This involves removing weeds by pulling or cutting them by hand. It is important to remove weeds before they produce seeds and spread further.
b. **Use of mulch**: Applying a layer of mulch around the *Paulownia* seedlings can help suppress weed growth. Mulch can be made from organic materials such as straw, wood chips, or dried leaves. This layer of mulch will prevent light from reaching the weeds and reduce competition for resources (Beckjord et al., 1985).
c. **Use of herbicides**: In the case of severe weed infestation, the use of herbicides may be an option. It is important to use herbicides responsibly and follow the manufacturer's instructions. Consult a weed control specialist or agronomist for specific recommendations and to ensure the correct and safe use of herbicides.
d**. Competition through plant density**: Another weed control strategy is to plant *Paulownia* seedlings at a higher density. By filling the available space with seedlings, weed growth space can be reduced, and excessive competition between them can be avoided. It is important to pay attention to weed control in the early stages of *Paulownia* seedling development, as it can significantly influence the success of the crop. Constantly monitor the plantation and take preventive or curative measures to effectively control weeds.
Consult a weed control specialist or agronomist for specific advice and appropriate recommend-dations for weed control in *Paulownia* seedlings, considering local conditions and specific crop requirements.

Technical cutting in *Paulownia* is an essential aspect of care and management in this crop. This practice aims to shape and control tree development to achieve healthy growth, proper form, and optimal timber production. One of the main objectives of technical cutting is to ensure proper branching of the trees. By cutting the shoots that appear in the leaf axils, the development of the stem is encouraged, and height and form are controlled. This contributes to achieving well-developed trees with a dense and uniform crown. Technical cutting is usually carried out during the vegetative dormancy period, before active growth of the trees begins in spring.
It is recommended to remove dry, damaged, or diseased branches to prevent the spread of diseases and maintain overall tree health. Additionally, technical cutting can be used to control plant density in the plantation. By selectively removing some seedlings, adequate growth space can be ensured for each tree, avoiding excessive competition for available resources. This practice is particularly recommended for plantations aimed at producing firewood.
Consulting a specialist in the field can be very helpful in performing correct and efficient technical cutting. In conclusion, technical cutting in *Paulownia* is an essential practice for achieving healthy growth, proper form, and optimal timber production. By correctly applying technical cutting, the success and profitability of *Paulownia* cultivation can be ensured.
Technical cutting is visible in Figure 3.

Figure 3. *Paulownia* technical cutting

These are just a few of the agrotechnical operations necessary in a *Paulownia* plantation. It is important to adapt the practices to the specific conditions of the soil and local climate and consult experts in the field to achieve optimal results.
Exceptional achievements visible in Figure 4 and Figure 5.

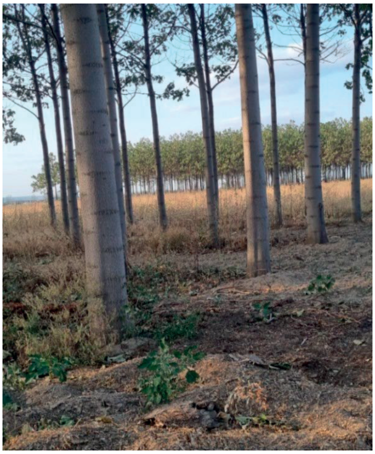

Figure 4. *Paulownia* plantation in Romania

Figure 5. *Paulownia* timber/lumber

The results obtained during the experimental period have highlighted differences between variants, determined by genotype and water administration. To quantify the extent to which the experimental factors influenced the height and diameter growth of *Paulownia* plants, both the cumulative and separate influences of the experimental factors were analyzed (Humenik et al., (2023). The evaluated biometric data represent the growth increments in height and diameter recorded in the autumn of 2023 for the shoots received in the spring of 2022, at 5 cm above ground level (Rad et al., 2015). The growth increment in height over the two years under the cumulative influence of the experimental factors (Table 1) indicates highly significant, positive differences in the irrigated variants. However, the exceedances compared to the control are within a wide range, ranging from 116.43% to 163.62%, with the highest values corresponding to the 'Cotevisa 2' hybrid (V_4). The non-irrigated variants (V_1 and V_3) had growth increments below the control level, with a decrease of 14.55 % for the 'Z07' hybrid and 110.90 % for the 'Cotevisa 2' hybrid.

Table 1. Cumulative influence of experimental factors on height growth

Variants	Height (cm)	% from x̄	Diff. from x̄	Signif.
V_1	239.00	52.70	-214.50	000
V_2	528.00	116.43	74.5	xxx
V_3	305.00	67.25	-148.5	000
V_4	742.00	163.62	288.5	xxx
Average (x̄)	**453.5**	**100**	**-**	**control**
LSD 5%= 3.50; LSD 1% = 5.20; LSD 0.1% = 8.40				

Similarly, in the case of stem diameter, with values ranging from 7.10 to 19.20 cm, the cumulative influence of the experimental factors in the years 2022 and 2023 (Table 2) resulted in highly significant differences compared to the control group. Positive differences were observed in the irrigated variants (V_2, V_4), while negative differences were observed in the non-irrigated variants (V_1, V_3). The maximum diameter (19.2 cm) was recorded in the stems of the irrigated 'Cotevisa 2' hybrid.

Table 2. Cumulative influence of experimental factors on diameter growth.

Variants	Diameter (cm)	% from x̄	Diff. from x̄	Signif.
V_1	7.10	57.46	-5.30	000
V_2	14.20	114.72	1.80	xxx
V_3	9.00	72.88	-3.40	000
V_4	19.20	154.94	6.80	xxx
Average (x̄)	**12.40**	**100**	**-**	**control**
LSD 5% =0.20; LSD 1% = 0.30; LSD 0.1% = 0.50				

To determine the separate influence of each experimental factor, the variants were grouped based on the factor being analyzed. Thus, the influence of genotype was evaluated by comparing the results obtained for each hybrid in both the irrigated and non-irrigated variants, resulting in two groups: Group I (V_1 and V_3) and Group II (V_2 and V_4). From Table 3, it can be observed that genotype strongly influenced the height growth capacity of the plants, with a positive effect determined by the 'Cotevisa 2' hybrid (V_2+V_4), but a negative effect of the 'Superhibrid 'Z07' hybrid.

Table 3. Influence of genotype on height growth.

Variants	Height (cm.)	% from x̄	Diff. from x̄	Signif.
V_I (V_1+V_3)	383.50	84.56	-70.00	000
V_{II} (V_2+V_4)	523.50	115.44	70.00	xxx
Average (x̄)	**453.50**	**100**	-	**control**
LSD 5% =6.60; LSD 1% = 15.20; LSD 0.1% = 48.30				

Genotype had a similar effect on stem diameter as well (Table 4), with larger diameter observed in the 'Cotevisa 2' hybrid and smaller diameter in the 'Z07' hybrid.
However, unlike height growth, the influence was relatively smaller, with the differences compared to the control being significantly distinct.

Table 4. Influence of genotype on stem diameter growth.

Variants	Diameter (cm.)	% from x̄	Diff. from x̄	Signif.
V_I (V_1+V_3)	10.70	86.29	-1.70	00
V_{II} (V_2+V_4)	14.10	113.71	1.70	xx
Average (x̄)	**12.40**	**100**	-	**control**
LSD 5% = 0.40; LSD 1% = 0.90; LSD 0.1% = 2.80				

The influence of the second experimental factor (irrigation) involved grouping the non-irrigated variants (V_1 and V_3) and the irrigated variants (V_2 and V_4), regardless of genotype. Both height growth and stem diameter indicate statistically significant differences compared to the control group (highly significant).
The height of plants in the irrigated variants recorded an average of 635,00 cm, which is 40% higher than the control value, compared to the non-irrigated plants, which did not exceed 300,00 cm (272 cm) (Table 5) (Ptach et al., 2017).

Table 5. Influence of irrigation on height growth

Variants	Height (cm.)	% from x̄	Diff. from x̄	Signif.
V_I (V_1+V_3)	272.00	59.98	-181.50	000
V_{II} (V_2+V_4)	635.00	140.02	181.50	xxx
Average (x̄)	**453.50**	**100**	-	**control**
LSD 5% =2.50; LSD 1% = 5.70; LSD 0.1% = 18.20				

The growth increment in diameter and height of the *Paulownia* hybrids was also studied for each of the three analyzed years. While the growth increment in height showed large variations from one year to another (Figure 6), the diameter remained within close values, especially in the irrigated variants of both hybrids (Figure 7).

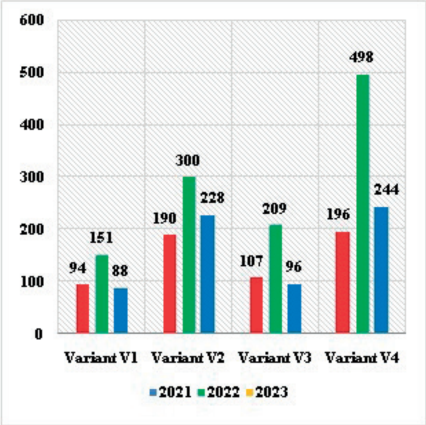

Figure 6. Growth increment in stem height (cm).

Similarly, in the case of stem diameter (Table 6), it was observed that irrigation led to a doubling of the stem diameter compared to the non-irrigated variants.

Table 6. Influence of irrigation on diameter growth

Variants	Diameter (cm.)	% from x̄	Diff. from x̄	Signif.
V_I (V_1+V_3)	8.10	65.32	-4.30	000
V_{II} (V_2+V_4)	16.70	134.68	4.30	xxx
Average (x̄)	**12.40**	**100**	-	**control**
LSD 5% = 0.10; LSD 1% = 0.30; LSD 0.1% = 1.10				

It should be mentioned that the values corresponding to the year 2021 represent the growth of the main stem in the first year from the cuttings used to establish the experimental crops. These stems were shortened to 5 cm in the spring of 2022. Therefore, for the years 2022 and 2023, the values correspond to the directed shoot as the main stem after the technical cut. It is evident that the maximum growth increments in height are achieved by the new shoot, resulting from the technical cut in the second year of experimentation (2022).

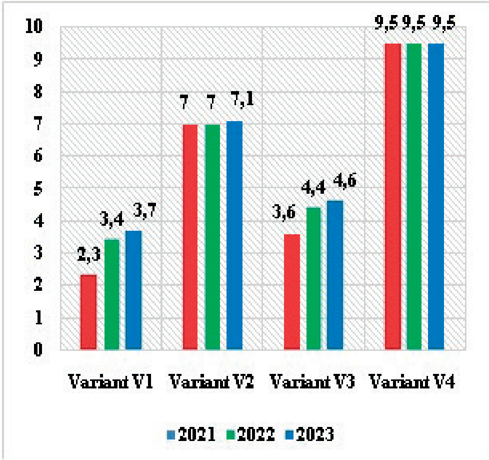

Figure 7. Growth increment in stem diameter (cm)

CONCLUSIONS

Based on the recorded and analyzed data, the following conclusions were drawn at the end of the preliminary analysis period:

1. A very good growth percentage was achieved in the 'Cotevisa 2' variety, showing an increase of over 20% compared to the Superhibrid Z 07 variety.
2. The use of irrigation systems in both varieties resulted in a 200% increase in annual development. Additionally, it was observed that the plants were not affected by phytosanitary diseases. The final success of the crop will depend on how all aspects related to plant care are applied and managed.
3. Stem height was the characteristic that showed the largest differences compared to the control group.
4. The application of technical cut to the main stem in the second year of vegetation resulted in greater vigor of the new shoots, with significant growth in height and diameter.
5. In terms of growth yield in height and diameter of the stems, irrigation is recommended for *Paulownia* crops, with better results observed in the 'Cotevisa 2' hybrid.

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CURRENT STATE OF RESEARCH ON BIOLOGICAL, ORNAMENTAL AND UTILITARIAN PARTICULARITIES OF SOME SPECIES OF THE GENUS *SALVIA* L. (LAMIACEAE)

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Abstract

This paper is a synthesis of research on the history and current state of knowledge regarding the biological, ornamental and utilitarian characteristics of some species of the genus Salvia L. (Lamiaceae). The analysis aimed to identify the literature from the Romanian and international academic literature, in which the genus Salvia L. (Lamiaceae) is treated, paying particular attention to the species Salvia officinalis, Salvia nemorosa, Salvia splendens, Salvia mellifera. During the activity carried out, historical milestones were determined and highlighted from the perspective of the scientific treatment of the genus Salvia, and a deeper dive into the already existing knowledge has been attempted, focusing on analyzing and capitalizing on the identified specialized works in which the biological, utilitarian and ornamental peculiarities are addressed. The results of the research highlight the importance of the species studied, as they may have an important practical use, both for lovers of sage flowers, as a garden plant, and for researchers and professional growers, as a medicinal and/or honey plant.

Key words: *biological, decorative, medicinal, melliferous.*

INTRODUCTION

The name of the plant upon which this tesys is centred on establishing its main peculiarities (biological, ornamental and utilitarian), *Salvia*, comes from Latin and represents a derivation of the term “save”, a, its meaning is “the one who saves”. *Salvia* is known by several names, the most relevant of which are salvia, salvir, chalet and lament.

Thus, is easy to assume the fact that it has had an important presence and use in the medical field, since antiquity. The importance of sage was not limited to the medicinal area, it was also used in the food field or as an ornamental and melliferous plant.

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HISTORIC

From a historical perspective, a first scientific approach to the genre was made by English botanist George Bentham, in *Labiatarum Genera et Species: or, a description of the genera and species of plants of the order labiatae* (1832-1836).

Regarding Sage, in the antereferred paper it is noted that, „of this numerous genus, some representatives can be found in almost all the world, but several of its sections are specific to different parts. Thus, *Eusphace*, *Horminum*, *Æthiopis* and *Hemisphace*, belong to the Mediterranean region; some few only of the Drymosphaces and Æthiopiders extending to the eastward as far as the mountains of Cashmere and the Himalaya. The Plethiosphaces are mainly European, Nort Asiatic, and Nort African; two or three species are extending also down the coast as far as the Cape of Good Hope. The Hymenosphaces are about equally divided between the Mediterranean region and the Cape of Good Hope, one scpecies belonging exclusively to the Canary Islands.

Notiophace contains an South Asiatic and Australian one and one North African species, and Gymnosphace an Indian one. Microsphace, Calosphace, Echinosphace, and Pycnosphace are exclusively American - the two latter confined to California; and Heterosphace, although a very natural section, is to be met with in the Mediterranean region, at the Cape, in Japan, and in Nort Africa" (Bentham, 1832).

Salvia officinalis L., has been known since the time of the Greeks and Romans, being mentioned in the writings of Dioscorides and Galen (Barbu et al., 2006).

Later, *Salvia officinalis* is described in 1753 by Carl Linnaeus as being used in the food field and also for healing, being mentioned in several texts for its medicinal properties. It was also considered an immortal substance and mentioned in the works of Greek and Roman doctors as a herb with healing qualities. Hippocrates called sage "sacred plant" and in ancient texts there were sayings like "How men can can grow old who has sage in his garden" (Devansh, 2012).

THE CURRENT STATE OF KNOWLEDGE OF SAGE

Sage, part of the Lamiaceae family, comprises about 500 species.

In this family there are aromatic, herbaceous plants, rarely woody in the form of shrubs and sub-shrubs, with stalks and opposite leaves, simple unstriped. The flowers are zygomorphic, hermaphrodite, clustered in dihazial cimes reunited in vertices. The calyx is gamosepal, in many situations bilabiate; it has the gamopetal corola, bilabiate, androceum didinam, bicarpal gynaeceum, upper-position gamocarpal and tetrachene-type mericarpic fruits (Ciocârlan, 2009).

The scientific clasification of sage is presented in Table 1:

Table 1. Sage systematics

Kingdom	Plantae
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Salvia*
Species	*officinalis, nemorosa, splendens, mellifera* (…)

In Romania, the presence of several species has been identified, among which we mention:

- species of culture (*Salvia officinalis*, *Salvia splendens* and *Salvia sclarea* L. – Serlai, last present also in spontaneous flora);
- spontaneous species (*Salvia. glutiesa* L., Cincest – shady places, forests, *Salvia. pratensis* L., field sage, *Salvia nemorosa* L., *S. nutans* L., *Salvia austriaca Jacq*, *S. verticillata* L., ear of the pig, all the latter from open places);
- endemic species in Romania - *Salvia transilvanica* Schur (Barbu et al., 2006).

According to Sin et al. (2001), wailing (sage) is among the species that are cultivated for herbs, leaves, flowers, inflorescences, roots and rhizomes (Sin et al. 2001).

Biological features

Barbu et al., 2006 in *Preliminary studies on the biological culture of sage* (*Salvia officinalis* L.), presenting the peculiarities of the genus *Salvia*, shows that „is a semi-shrub of the Labiatae family, thick, with straight or inclined shoots, covered with a brown to light gray bark.

The root is a lignified and highly branched rhizome, the ramifications penetrating deep.

The semi-lignified stem has 25-80 cm high branches, almost round, covered with a felt layer of gold-colored hairs – whitish. The opposite arranged leaves have a 1-5 cm long petiole, oval to elliptical or lanceolate shape. The color varies from white to gray for young plants, to green to gray for the mature ones. It presents a fine pubescence with multicellular hairs (1-4). The characteristic glands of Labiatae in most cases consist of 8 secretory cells.

The inflorescence is simple or branched, spiciform, made up of pseudo-verticillate with 3-10 flowers placed at the branch of the last 5-8 pairs of leaves, the green calyx – violaceus, consisting of 2 lips. The corolla is bilabial, double the length of the calyx, with the lower labium trilobate, longer than the upper one and with the middle lobe bent downwards. Androceum has 4 stamens of which 2 anterior are longer and fertile representing true stamens, the other 2 are short, rudimentary.

Fruits are dark tetrachaenium in the persistent calyx, brownish, ovoid, small 2-3 mm (of them only 1-3 reach maturity).

The seeds are round, dark; the average weight of 1,000 seeds is about 7-72 g, and 129 seeds per gram. Flowering begins in the second year, and the economic duration of a crop is 8-10 years, the semi-shrub growing up to 12-15 years, but with low vigor.

The entire plant emits a strong characteristic smell with a bitter aromatic taste (Barbu et al., 2006).

In the genus *Salvia*, *Salvia officinalis* is a perennial species, 30-70 cm high, showing oval leaves, cordate-hastate-sagitated base, with sharp basal lobes, as well as leaf tips, vertices with 2-6 yellow corolla flowers, sometimes stained with brown (Ciocârlan, 2009).

Ornamental features

Within the species *Salvia officinalis* we identify the form of *albiflora* Schur (white flowers with green calyx), with varieties such as:

- *salicifolia* (wide and short leaves), *icterina* (green leaves stained with yellow), *aurea* (golden leaves), *purple* (intense red leaves), *tricolour* (leaves showing the turning of the colouring from grayish green and white-yellow to intense red), used, having regard to the variety of shapes and the different colouring of the leaves, in the ornamental field, on calcareous, sunny lands, in curbs, on rocky places, in ruins;
- *argentea* (having strong-flavoured leaves) and *spinosa* (high oil content), used predominantly in crops for production (Barbu et al., 2006).

Another species of the ornamental relevant genus is *Salvia nemorosa* (Figure 1), an attractive-looking plant with a wide presence in gardens since the distant past, easy to grow and with many varieties and hybrids, it was described by Carl Linnaeus in 1762 as *nemorosa* ("of the forests") referring to its typical habitat in the grove and forest (Clebsch et al., 2003).

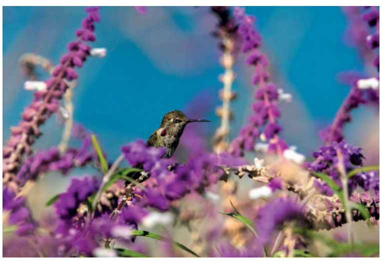

Figure 1. *Salvia nemorosa* (Roberto So)

Also, *Salvia splendens* (Selow - Garden Wales, scarlet sage) (Figure 2), blue-violet, white or yellow, with flowers grouped by (15)20-40 in dense verticals, oval-triangular leaves, with a corded or triangular base and 1-2 pairs of segments at the base, in consideration of its aesthetic qualities, is widely cultivated as an ornamental plant (Ciocârlan, 2009).

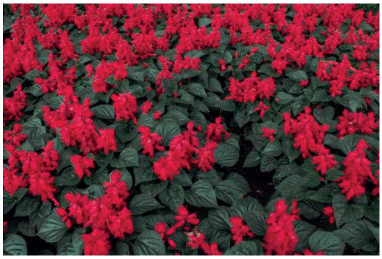

Figure 2. *Salvia splendens* (Murillo Molissani)

Sage splendens, native to Brazil, features a multitude of selected varieties and different colors. It does not survive freezing temperatures, but it can grow in cold climates as an annual plant. The most common selections encountered are dwarf-sized ones ('Sizzler' and 'Salsa'), mass-planted in gardens and malls. 'Van Houttei' reaches the height of 1 to 1.3 m (Clebsch et al., 2003).

Recently introduced in the Romanian culture for its ornamental role is *Salvia farinacea*, perennial plant - annual in Romania (Badea, et al., 2008).

Salvia farinacea is native to Nuevo Leon, Mexico United States (Texas and Oklahoma) with spectacular purple-blue peaks (Turner, 2008).

Utility features

The economic importance of sage, as we deduct from its very name, is not limited to its use only in relation to its aesthetic qualities.

In this regard, we consider, first of all, its usefulness as a medicinal plant, as well as, why not, as a melliferous plant or which is used in the food field.

"Sage (*Salvia officinalis*) has been conventionally used for the treatment of various illnesses since ancient times in various parts of the world. The immense usage of sage, including in the culinary, medicinal, aromatic and pharmaceutical fields makes it the most popular among essential oil-bearing plants. With an increasing demand over good quality organically grown sage, there is a need for further research to standardize the organic cultivation techniques including seed priming to economically viable extraction and processing methods. The quality of the herb and essential oil content is highly variable depending on environmental conditions and harvesting time. Findings from in-vitro and several clinical studies establish the evidence of its medicinal uses such as cognitive, anti-diabetic, hypo lipidemic, anti-cancer and anti-inflammatory properties. Though it is a very potential and commonly available herb for pharmaceutical industries, there is a need to develop extraction and isolation protocols for testing the efficacy of the herb. The various biologically active compounds in sage are responsible for its pharmacological properties and the extent of cytotoxicity are still unknown and hence there is a lot of scope to conduct drug dosage studies in the future." (Yashaswini et al., 2019).

Therefore, a decisive aspect for the utility of sage lies in its chemical constituents. In Table 2 and Table 3 we present, on the one hand, the chemical constituents of *Salvia officinalis*, as well as the concentration of polyphenols and phenols in hydroalcoholic extracts expressed in µg/g.

Table 2. Chemical constituents of S. *officinalis* (Mehta Devansh, 2012)

Moisture	5.7%
Total Ash	7.7%
Sodium	0.01%
Vitamin B2	0.34mg/100 g
Protein	10.2%
Calcium	1.8%
Potassium	1.0%
Vitamin C	39.8mg/100 g
Fiber	16%
Phosphorous	0.09%
Vitaim A	2395 I.U./100 g
Niacin	5.7mg/100 g
Carbohydrates	46.3%
Iron	0.03%
Vitamin B1	0.75mg/100 g
Caloric value	415 calories/100 g

Table 3. The concentration of polyphenols and phenols in hydroalcoholic extracts of *Salvia officinalis* expressed in µg/g (Neagu et al., 2014)

Gallic acid	0.85
Chlorogenic acid	0.50
Caffeic acid	1.91
Rutin	1.30
Coumaric acid	4.50
Ferrulic acid	1.21
Rosmarinic acid	17.25
Myricetin	1.45
Quercetin	2.80

The usefulness of sage as a medicinal plant

As we mentioned before, Sage has had an important presence and use in the medical field since antiquity (Figure 3).

Figure 3. Sage has been used in medicine since antiquity (Huibre Venter)

Currently, it is recognized the possibility of using sage from a medicinal perspective, on the following levels:

- alternative and complementary medicine for primary dysmenorrhea, the essential oils of *Salvia officinalis* (linalyl acetate, linalool, eucalyptol and (Caryophyllene) being responsible for analgesic activity;
- antibacterial properties on gram-negative and gram-positive bacteria, the synergistic antibacterial activity of extracts of *Salvia officinalis* and *Cichorium intybus* being compared with antibiotics such as amoxicillin and chloramphenicol;
- anti-inflammatory activity, salvia-specific diterpenes acting as a potent anti-inflammatory phytoconstituent;
- anti-nociceptive activity, carnosol (10 mg/kg) and ursolic acid/oleanolic acid (30 mg/kg) inhibiting the inflammatory phase of the formol, nociception and cinnamaldehyde-induced mechanical allodynia;
- cancer therapy, being investigated free radical scavenging properties in plant extracts from *Salvia officinalis*;
- the antidiabetic potential. Investigating the antidiabetic potential of tuyona, a major constituent of the *Salvia* species, it was found to correct the lipid profile (cholestrol and triglycerides) in diabetic rats;
- antioxidant potential;
- Alzheimer's disease, following the research carried out by finding that *Salvia officinalis* is effective in treating the condition of the disease;

- improvement of dementia, specific phytoconstituents reducing, depending on the dose, the pathological conditions leading to dementia;
- antimicrobial and antifungal activity, being studied the inhibitory effect of essential oils from sage leaves (*Salvia officinalis* L.). It was found effective inhibition of *S. aureus, E. coli, Salmonella;*
- viral pharyngitis, as a result of the studies carried out, the effectiveness of plant extracts from *Salvia officinalis* L. in viral pharyngitis, 15% sage spray% providing a convenient and safe treatment for patients with acute pharyngitis;
- management of gum and periodontal diseases, extracts of *Salvia officinalis* L. significantly improving the gingival index and score of the plaque index. Devansh, 2012; Yashaswini et al., 2019).

The usefulness of sage as a melliferous plant

Salvia mellifera (California black sage) produces a nectar from which black sage honey is made, a rare honey, very spiced, which can be created only under certain specific conditions (Figure 4).

On the importance of the usefulness of sage in the medical field are also of interest the records of Romero (1954) on the use of American black sage (*Salvia mellifera*) for healing long-term deep dry cough, which has settled in the bronchial tubes (Romero, 1954).

Figure 4. Sage, melliferous plant (Janice Carriger)

There are also studies highlighting the usefulness of black sage (*Salvia mellifera*) to relieve chronic pain. In this respect it is relevant that black sage, which contains 54 monoterpenoids and several diterpenoids, such as carnosolul (41%), acidul carnosic (22%), salvicanolul (15%) și rosmanolul (9%) [3,4]. Monoterpenoids are 1,8-cineole (39,8%), camphor (12,2%), α-pinene (9,2%), limonene (2,2%), myrcene (2%), γ-terpinene (2%), terpene-4-ol (2%) and many less abundant monoterpenoids, it is considered the traditional medicine of the Chumash Indians of California (Adams et al., 2019).

The usefulness of sage as an edible plant

Sage appears mentioned by *Le Viandier de Taillevent* in the fourteenth century, in a culinary recipe that bears his name – *Cold sage sauce* (Prescott, 1948).

Also, as we have shown in the History section, *Salvia officinalis* is described in 1753 by Carl Linnaeus as being used in the food field (Devansh, 2012).

Currently, Chia seeds (*Salvia hispanica* L.) are increasingly being used by food processors and consumers, with a predominance for food fortification. In this context, in studies conducted to determine the mineral composition of chia seeds, it was found that they contain, depending on the planting season and the region in which they are grown, phosphorus (531 to 889 mg/100g), calcium (478 to 589 mg/100 g), potassium (343 to 526 mg/100 g) and magnesium (322 to 440 mg/100 g), which highlight them as relevant in fortifying food (Figure 5) (Pauline et al., 2023).

Figure 5. Chia - *Salvia hispanica* (Charlotte May)

Sage, naural psychedelic

Salvia divinorum it is a natural psychedelic considered one of the most powerful hallucinogens discovered to date. Few behavioral studies have concluded that the effects of sage may be similar to those of traditional psychedelics, which is noteworthy because sage acts through a unique molecular mechanism as an agonist of the kappa opioid receptors. One hundred and ninety-three participants, including 34 users of sage, were asked to complete a series of questionnaires related to the general drug use, personality characteristics, and, demographics and their experiences with sage. Sage users were found to differ from non-users in terms of personality characteristics and reported consuming significantly more alcohol than non-users. In addition, although users of sage rated their hallucinogenic experiences as similar to those seen in previously published reports, most have compared their experiences to being more marijuana-like instead of more traditional psychedelics. The low scores at the ARCI LSD sub-scale confirmed this finding and call into question the reigning theory of LSD-like subjective effects caused by Salvia. (Albertson et al., 2009).

CONCLUSIONS

Sage has multiple uses, most in the field of medicine, but also, not at all negligible, in ornamental, culinary and melliferous field.
Given the social impact, as well as the extended economic value, in the field of medicine, this utility of sage has been the subject of several specialized analyzes, fact also revealed in the research identified and highlighted in this paper. However, it is also not to be neglected the other areas where sage can be exploited from an economic perspective.
We consider, in particular, the usefulness of sage as an ornamental plant, which stems from its important aesthetic characteristics (it is a decorative plant both through leaves and flowers, which have a special and intense color and long flowering duration during the year). These characteristics, if more highlighted and presented to interested subjects, could lead to a more important use in residential and urban landscaping.
Moreover, sage attracts insects (bees, butterflies, etc.), which are beneficial to ornamental or production gardens.
At the same time, it is drought resistant, being ideal for hot and dry areas, including for gardens where there are no or cannot be implemented irrigation systems.
Also, the simplicity of gardening-specific activities that are required to be carried out for the maintenance of this species makes it the ideal choice for beginner or limited-time gardeners.
In this context, we note the need to expand, on all levels where they can be carried out, the research to emphasize and, at the same time, highlight the usefulness of sage in the ornamental field.

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STUDY ON THE USE OF PAPER WASTE AS AN ALTERNATIVE SUBSTRATE FOR *FICUS BENJAMINA* SPECIES

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Abstract

The main objective of the paper was to establish the effectiveness of paper waste added to a growth medium, avoiding waste management processes, which require time and energy, but providing an efficient solution for using paper waste as a nutrient substrate for plants cultivated in containers. It was specifically aimed to evaluate the percentage of peat that could be replaced by waste paper for the ornamental plant Ficus benjamina cv. 'Golden King' in an experiment with 4 variants in 3 repetitions (V1 - 100% peat, V2 - 80% peat+20 % paper waste, V3 - 70% peat+30% paper waste, V4 -50% peat+50% paper waste) in 2023. In conclusion, based on the research carried out, it can be stated that paper waste mixed with peat in a proportion of 30% can be used as an alternative substrate.

Key words*: paper, waste, alternative substrate, peat.*

INTRODUCTION

The practice of using waste paper for horticultural plant production is considered an alternative of peat after composting (Molitor & Bruckner, 1997) and has gained popularity in recent years due to its environmental benefits.
One challenge in finding a suitable peat substitute is ensuring that the growing medium can support optimal plant growth without the need for additional fertilizers or pesticides (Pasquier et al., 1982).
Research is ongoing to develop new, sustainable substrates for horticulture that can replace peat while maintaining plant health and productivity. This includes investigating novel materials as well as studying the interactions between plants and different growing media.
By using waste paper as an alternative substrate, we can reduce the demand for peat extraction, which is a non-renewable resource that is often harvested unsustainably from natural peatlands (Hirschler et al., 2022).
Composted waste paper can provide a valuable source of organic matter and nutrients for plants, helping to improve soil structure and fertility. Additionally, using waste paper for horticultural purposes can help to divert this material from landfill, reducing the amount of waste contributing to pollution and waste valuable resources, minimizing costs for both industry and the environment (Vannucchi et al., 2020).
In 2020, a quantity of paper and cardboard of 32.7 million tons was generated in the EC member countries, which represents 41.2% of packaging waste. The highest recycling percentage of paper and cardboard waste was recorded in Belgium at 79.2%, followed by Norway (78.8%) and Luxembourg (71.9%), while in Romania the recycling percentage was of 44.6%) (***, Eurostat, 2021).
35 % of the felled trees cut worldwide are used for paper production, paper consumption has increased with 400 % over the last 40 years globally (Cheung & Pachisia, 2015) and due to the fact that single-use plastic products have been replaced by paper and cardboard ones. It is considered that paper is contributing to 31% of the global packaging market (Jones & Comfort, 2017).
Overall, using waste paper for horticultural plant production is a sustainable and environmentally-friendly practice that can help to conserve natural resources and reduce waste. It offers a viable alternative to peat and can contribute to the circular economy by closing the loop on paper production and consumption.
Both recycling and the recovery of paper and cardboard with energy recovery bring benefits

in terms of reducing greenhouse gas emissions and of course significant benefits to landfills (***, H.G. 870/2013).

The results of the study done by Jones et al., 2020, suggest that composted waste paper has the potential to be a valuable resource in agriculture, helping to improve soil quality and support plant growth.

The research done by Chrysargyris et al., 2018, found that using 30% paper waste as a substitute for peat in potting culture for marigold and petunia did not have any negative effects on plant growth or development. However, when they tried to use the same substitution rate for *Matthiola*, they found that the physicochemical properties of the substrate needed further improvement in order to support optimal plant growth.

This study suggests that while paper waste can be a viable substitute for peat in certain potting cultures, it may not be suitable for all plant species without additional adjustments to the substrate (Chrysargyris et al., 2019).

The addition of paper waste can increase the organic matter content and lowered the pH of the substrate. These changes can have both positive and negative effects on plant growth, depending on the specific needs of the plant species being grown. Additionally, the altered physicochemical characteristics of the substrate may impact the overall health and stability of the plant ecosystem in which the substrate is used (Chrysargyris et al., 2020).

MATERIALS AND METHODS

This study was undertaken to evaluate the suitability of paper waste as growing media for *Ficus benjamina* cv. 'Golden King' plants cultivated in containers and it was specifically aimed to evaluate the percentage of peat that could be replaced by waste paper. The study involved the establishment of experimental containers with varying percentages of paper waste mixed with peat, as a growth medium and the characterization of the components that were used for the nutrient mixture through physical and chemical analyses.

The morphological characters such as plant height, length of shoots, length of leaves, width of leaves, length of internodes, and number of shoots were measured over a period of time of 4 months.

At the end of the experimental period, determinations were made on the root and aerial parts: number of roots, root length (cm), root diameter (cm), root weight, aerial part weight, and total plant weight (g).

This research study was done at the greenhouses of the Botanical Garden A.I. Buia, Craiova. The temperature in the greenhouse was 20-22°C and humidity between 60-80%.

Paper waste, used as an alternative substrate, came from A4 paper, which was cut into paper strips with a length of 3 cm and a width of 0.5 mm, the biological material used was constituted by the *Ficus benjamina* species, cv. 'Golden King', species of decorative plant through leaves in the *Moraceae* family, native to Asia and Australia.

The experimental scheme (Table 1) had 4 variants, and each variant of the experimental scheme had 3 repetitions. Peat and paper waste were mixed in different percentages according to Table 1 in plastic containers with a diameter of 9 cm and a volume of 0.45 l, which has a capacity of 105 g of substrate.

Table 1. Experimental scheme

Variants	Growth media
V1(control)	100% peat
V2	80% peat+20% paper waste
V3	70% peat+30% paper waste
V4	50% peat+50% paper waste

The peat used as substrate in the containers is a fine Baltic white peat, free from weeds, pests and diseases, with an average diameter size between 0-20 mm and has the following chemical composition shown in Table 2. Peat fertilizer (N.P.K. + trace elements) provides a sufficient level of nutrients for 3 to 6 weeks.

Table 2. Chemical composition of peat substrate

Parameter	Value
pH	5.8
EC (mS/cm)	0.112
Organic matter (%)	79
N (%)	4.6
P (%)	0.9
K (%)	2.5
Ca (%)	4.2
Mg (%)	1.4
Fe (mg/kg)	3000
Zn (mg/kg)	90
Cu (mg/kg)	20
Mn (mg/kg)	80
Mo (mg/kg)	5
Density (kg/m^3)	171.1

The physico-chemical analyzes of the substrates from the 4 variants were carried out according to the following methods:
- pH in distilled water, potentiometric determination 1:4;
- humus (H%), Walkley-Black method modified by Gogoasa;
- total nitrogen (N%) - Kjeldahl method;
- mobile phosphorus (P_{AL}), Egner - Riehm - Domingo method, spectrophotometry, λ = 660 nm;
- mobile potassium (K_{AL}) extraction with ammonium acetate lactate similar to P_{AL} by flamephotometry;
- electrical conductivity (EC), conductometric determination 1:5 dilution method;
- organic matter determined by calcination;
- Mg, Cu, Fe spectrophotometric determination.

The interpretation phase consisted in the interpretation of analytical data, their correlation with field determinations and observations. The experimental data for plant growth parameters, were analysed using one-way analyses of variance (ANOVA) and Tukey's post-hoc test at a significance level of 0.05 performed by using the MINITAB statistical package (Minitab, State College, PA, USA). The experimental values are presented as the mean of three replicates.

RESULTS AND DISCUSSIONS

The physico-chemical composition of the substrate (peat-paper waste) is presented in Table 3.

Table 3. Physico-chemical composition of the substrate (peat-paper waste)

Parameter	Variant			
	V1	V2	V3	V4
pH	5.8	5.95	6.12	6.24
EC (mS/cm)	0.112	0.197	0.230	0.323
Organic matter (%)	79	78	78	76
N (%)	4.83	3.93	3.492	2.6
P (%)	0.94	0.758	0.696	0.489
K (%)	2.62	2.144	1.906	1.431
Mg (%)	1.47	1.176	1.038	0.751
Ca (%)	4.41	3.564	3.141	2.295
Fe (mg/kg)	3150	2537.18	2230.77	1617.95
Zn (mg/kg)	94.5	78.18	70.02	53.7
Cu (mg/kg)	21	23.72	25.08	27.8
Mn (mg/kg)	84	68.38	60.84	45.4
Mo (mg/kg)	5.25	4.2	3.67	2.625

According to Chrysargyris et al., 2019, the chemical composition of paper waste is as follows: electrical conductivity of 1.175 mS/cm, pH of 6.31, organic matter 75.52%, nitrogen 0.37%, potassium 2428.5 mg/kg, phosphorus 389.2 mg/kg, Ca 1804.8 mg/kg, Mg 332.7 mg/kg, Fe 85.9 mg/kg, Cu 34.6 mg/kg.

The addition of paper waste in the growth substrate in percentages of 20%, 30% and 50% leads to an increase of pH value from 5.8 for variant V1 (moderately acidic reaction) to 6.24 for V4 (weakly acidic reaction), an aspect also observed by Chrysargyris et al., 2019.

Increasing the pH value in the weak acid range leads to greater solubility and mobility of nitrogen, phosphorus, potassium, copper and zinc. The absorption of nutrients, with the exception of molybdenum, is greatly favored by the slightly acidic reaction (Rusu et al., 2005).

The electrical conductivity increases in all variants, registering the value of 0.112 mS/cm in V1 and reaching 0.323 mS/cm in V4, EC being in all 4 variants below the value of 0.5 mS/cm which is the optimal value for substrates (Fascella, 2015; Zulfiqar et al., 2019).

According to Craig & Cole, 2000, electrical conductivity values between 0.1-1.8 mS/cm and pH values between 5.0 and 6.5 indicate a normal availability of nutrients for plants, all values recorded for all variants being between these limits. Peat is widely used in growing plants in containers because most of the nutrients are easily available to the plants (Vannucchi et al., 2020).

The content of macroelements and microelements decreases from V1 to V4, with the exception of copper, which registers an increase from the value of 21 mg/kg (V1) and reaches the value of 27.8 mg/kg (V4). This increase in copper can be explained by the high content of copper in the paper used for the substrate.

Figure 1. Summary images during experimental research

The mobility of copper is dependent on pH (Dodocioiu et al., 2009), as can be observed from Table 3, the copper content increasing as the pH increases.

In a strongly acidic environment, copper cations accumulate the most in the root system of plants and less in other organs, and the movement of the soil reaction towards the pH = 6.8-7 range increases their translocation and accumulation in the aerial organs (Rusu et al., 2005).

Table 4. Effect of different culture substrates on vegetative growth parameters at the end of experiment

Variants	Height of plants (cm)	Length of shoot (cm)	Length of leaf (cm)	Width of leaf area (cm)	Length of internode (cm)	Number of shoots
V1 (Co)	16.27a	1.76b	7.00a	2.33a	2.13a	13.67a
V2	13.23a	2.50a	5.73ab	2.36a	2.26a	11.67a
V3	9.20b	1.60b	5.60ab	2.30ab	1.80a	6.00b
V4	7.50b	1.50b	4.43b	1.93b	2.00a	4.67b
F test	**22.46***	**8.10***	**5.66***	**5.43***	**1.11**	**32.38***

Means comparison were done using Tukey's test ($p<0.05$). Different letters indicate statistically significant differences between treatments.

In *Ficus benjamina*, the addition of paper waste reduced vegetative growth (plant height, shoot length, leaf length, leaf width, internode length, number of shoots), while the addition of 20% paper waste caused an increase in the average length of the shoots and the average length of the internodes compared to the other analyzed variants.

The addition of paper waste in the *Ficus benjamina* culture substrate caused a significant decrease in the average height of the plants in the variants where 30% paper waste (V3) and respectively, 50% paper waste (V4) were used, compared to the control variant (V1 - 100% culture substrate) and with variant V2 - 20% paper waste.

There were no significant differences between the control variant (V1 - 100% culture substrate) and V2 - 20% paper waste, nor between the variants V3 - 30% paper waste and V4 - 50% paper waste.

Given that rapid plant growth is not advantageous for the majority of ornamental plants (Manda et al., 2008), the addition of paper compost to the growing substrate could prove beneficial, with the potential effect of maintaining or even reducing plant size.

The average length of shoots, after 4 months from the start of the experiment, recorded the highest value at V2 - 20% paper waste, and the lowest value at V4 - 50% paper waste.

From a statistical point of view, there are significant negative differences between V2 and the other experimental variants.

The average length of the leaves recorded the highest value at V1 - 100% substrate, and the lowest value at V4 - 50% paper waste. From a statistical point of view, there is a significantly negative difference between V1-100% substrate and V4 - 50% paper waste, the differences between the other variants are not statistically ensured.

In the case of the average width of the leaves, only V4 (1.93 cm) recorded a significant decrease compared to V1 (2.33 cm) and V2 (2.36 cm) (Table 4).

For plants grown in pots, the characteristics of the leaves are an important parameter, because they give a compact appearance and commercial value to these plants (Nicu & Manda, 2023).

The addition of paper waste in the culture substrate did not have a significant influence on the average length of the internode, the values being between 1.80 cm (V3) and 2.26 cm (V2).

The average number of shoots per plant recorded a significant decrease in the average height of the plants in the variants where 30% paper waste (V3) and 50% paper waste (V4) were used compared to the control variant V1 - 100% culture substrate and with V2 - 20% paper waste. There were no significant differences between V1 - 100% peat substrate and V2 - 20% paper waste, nor between the V3 and V4 variants.

In Table 5 is presented the effect of different substrates on the variability of the morphological characters of the roots.

Table 5. Effect of different culture substrates on root morphology, aerial and root biomass in *Ficus benjamina*

Variant	Number of roots		Root length (cm)		Root diameter (cm)		Root weight (g)		Aerial weight (g)		Total weight plant (g)	
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV
V1(co)	5.33±0.57b	10.83	12.00±1.00a	8.33	7.66±0.47b	6.16	1.09±0.09b	8.75	5.93±0.30a	5.14	7.02±0.40a	5.70
V2	4.66±0.57b	12.37	16.63±0.35a	2.11	5.16±0.30c	5.91	0.89±0.06b	6.81	3.05±0.07b	2.54	3.68±0.52b	14.23
V3	8.33±0.57a	6.93	13.07±0.19a	1.52	9.30±0.20a	2.15	1.43±0.09a	6.74	2.14±0.07c	3.53	3.57±0.16b	4.48
V4	4.66±0.57b	12.37	10.03±0.15c	1.52	1.90±0.40d	21.05	0.94±0.04b	4.26	1.28±0.21d	16.63	2.22±0.25c	11.37

Analyzing the obtained data, it is observed that for root diameter, aerial weight and total weight of the plant, a large variability is recorded depending on the culture substrate.

The highest number of roots was recorded at V3 (8.33 roots), while the lowest number of roots was observed at V2 and V4 (4.66 roots), with the difference being statistically significant.

Significant differences were recorded regarding the diameter of the roots, the average values being in the range of 1.9 cm (V4) and 9.3 cm (V3), compared to the control V1, only V3 recorded higher values than this.

The average root weight reached its maximum value at V3 (1.43 g), which also corresponds to the highest number of roots, and the difference compared to the other analyzed variants is statistically significant.

Regarding the aerial part of the plant, the limits of variation are between 1.28 cm (V4) and 3.05 cm (V2), compared to the control variant which recorded a value of 5.93 cm. For this parameter, significant differences were observed between all variants, with a decrease in values as the proportion of paper waste increased.

The same trend is observed for the total weight of the plants, with the highest values recorded in the control variant (V1 - 7.02 g), and the lowest values corresponding to the variant with the highest addition of paper waste (V4 - 2.22 g).

CONCLUSIONS

The present study suggests that for the container culture of *Ficus benjamina*, the culture substrate can be replaced with up to 20% paper waste with an insignificant reduction of the main parameters that indicate vegetative growth, respectively the aesthetic value of the plants.

Given that rapid plant growth is not an advantage for most ornamental plants, the addition of waste paper to the growing medium could prove beneficial, with a potential effect of maintaining or even reducing plant height.

Using waste paper as a substrate for potted plants can be an ecological and economical alternative to conventional substrates. Recycled paper can be used to provide support and nutrients to plants, while helping to reduce the amount of waste that ends up in the environment.

Recycled paper can also have economic advantages as substrate welfare costs can be reduced compared to conventional substrates. By recycling paper waste to use as plant substrate, energy needed to produce new substrates can also be saved.

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ASYMBIOTIC SEED GERMINATION AND *IN VITRO* SEEDLING DEVELOPMENT OF PHALAENOPSIS ORCHID HYBRIDS

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Abstract

The commercial exploitation of Phalaenopsis, an epiphytic orchid, is widespread, but due to its challenging vegetative propagation, there is a necessity to enhance micropropagation techniques. This study aims to determine the optimal medium and growth regulators for the successful asymbiotic seed germination, development, and multiplication stages of Phalaenopsis. Seeds from 4-month-old-hand-pollinated orchid were sown on ½ Murashige and Skoog (MS). The resulting PLBs were transferred on 4 types of culture media:1/2 MS (1), Malmgren (MLG) media (2), ½ MS with MLG vitamins (3), ½ MS with MLG vitamins, microelements and iron at full strength (4).The fourth medium was found most suitable as it provided the highest length and overall best conditions for evolution. In the multiplication stage a variation of explants: protocorms, leaves, stems and roots were put on 3 culture media, with a variation of light condition, dark and 16/8 photoperiod. The first media did not contain growth regulators and the other two containing also 2 mg/l BAP +0.5 mg/l NAA+100 mg/lPVP+1 g/l CA, respectivly 2.5 mg/l BAP+0.75 mg/l NAA + 100 mg/l PVP + 1 g/l CA.

Key words: *Phalaenopsis, in vitro tissue culture, asymbiotic seed germination, seedling development, multiplication.*

INTRODUCTION

Comprising 62 species, Phalaenopsis stands as a multifaceted orchid genus primarily harnessed for commercial purposes, serving as both a cut flower and a potted plant. This orchid variety proves to be a lucrative venture in flower markets globally. The popularity of Phalaenopsis within the Orchidaceae family stems from its enduring flowers and the ease with which it can be cultivated in home environments (May, 2018).

Hybridization or cross-pollination within breeding programs has consistently proven to be a dependable method for generating a diverse array of successful cultivars. These cultivars exhibit appealing combinations of characteristics such as spray length, bud number, flower color and type, fragrance, seasonality, and compactness (Trevor, 2011). Over time, the orchid market has transformed from a hobbyist's pursuit to a highly commercialized industry, with considerable potential for further expansion (Edy Setiti, 2019). The horticultural sector is now witnessing a shift towards large-scale cultivation of orchid cut flowers and potted orchids. The ability to mass-cultivate orchids became feasible with a significant breakthrough in orchid seed germination, laying the groundwork for extensive breeding and the creation of new commercially viable orchid hybrids (Zheng, 2010).

Knudson's groundbreaking work in 1922 on asymbiotic germination of orchid seeds played a pivotal role in advancing plant tissue culture techniques for orchid micropropagation (Knudson, 1922). The development of asymbiotic seed germination and clonal propagation in vitro has not only facilitated large-scale orchid cultivation but has also established a viable economic market.

Given the minuscule size of orchid seeds, with a single fruit or capsule capable of producing up to a million seeds depending on the species, the potential for propagation is immense. However, due to their limited food storage, orchid seeds rely on a specific type of fungus from orchid mycorrhizae for germination and development. *In vitro* germination using nutrient media containing sugars has become crucial, particularly considering the low survival rate in the wild habitat (under 5%).

Orchid seeds stand apart from most flowering plants due to their tiny embryos. Unlike many plants, orchid seeds lack apical meristems and cotyledons at the time of seed dispersal, and they

exhibit diverse embryo developmental patterns, particularly in suspensor morphology. Orchid seeds have a unique germination process as they rely solely on fungal infection. Consequently, a practical method for orchid seed germination involving fungi has been employed for an extended period (Lee, 2007).
Following Knudson's revelation in 1946 that orchid seeds could successfully germinate on a straightforward medium consisting of minerals and sugar, the adoption of asymbiotic procedures became widespread, supplanting the previously favored symbiotic methods.
The efficacy of in vitro seed germination is affected by various factors, encompassing the nature of the culture media, seed maturity, plant growth regulators (Paudel et al., 2012), carbohydrates (Huh et al., 2016), and organic amendments.
The aim of this study was to determine the optimal medium and growth regulators for the asymbiotic seed germination, development, and multiplication stages of Phalaenopsis-type hybrids.

MATERIALS AND METHODS

1. Plant Material: Surface Sterilization of Capsule and *In Vitro* Asymbiotic Germination

The Phalaenopsis capsule, obtained from hybridization was kindly provided by Cătălina Nicolae, University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV). The four-month-old light-green capsule, hand-pollinated, underwent a process of immersion in 70% alcohol for 2 minutes, repeated four times, with intermittent rinsing using tap water.
Under the laminar-flow hood the capsule was immersed for 2 minutes in 70% ethyl alcohol, rinsed with distilled water. In the next stage, work was done with the help of a stirrer, for 15 minutes using a solution of 2% sodium dichloroisocyanurate (NaDCC) plus a jet of Domestos (cleaning solution based on chlorine, Romania). The capsule was left unrinsed, it was removed on a sterile filter paper to absorb the club. Later, it was cut transversely and longitudinally with a scalpel with a sterile blade. Work was done without touching the extremities. The small, powder-like seeds were sown with the help of a sterile scalpel on 1/2 Murashige and Skoog (MS) medium without added hormones, poured into plastic Petri dishes (Figure 1).
The seeds on the culture medium were maintained under a photoperiod of 16 hours of light per day, accompanied by 8 hours of darkness, at a temperature of 23 degrees Celsius ± 2.

Figure 1. Sowing the seeds with the help of a sterile scalpel on 1/2 Murashige and Skoog (MS) medium

2. *In Vitro* Protocorm-like Bodies (PLB) Development on Different Media

We employed protocorm-like bodies (PLBs) obtained from the preceding asymbiotic in vitro germination stage. Four different types of medium, devoid of added hormones, were assessed to determine the most advantageous for protocorm development: (1) half-strength Murashige and Skoog (Murashige & Skoog, 1962) (½ MS macro and micronutrients); (2) Malmgren medium (MLG) (Malmgren, 1996); (3) ½ MS with MLG vitamins and (4) ½ MS with MLG vitamins, microelements and iron at full strength. All media were supplemented with 30 g·L-1 sucrose, 1 g·L-1 Activated charcoal (AC) and solidified with 7 g·L-1 agar powder. pH was adjusted to 5.75 with 0.1M NaOH or HCl. Media were sterilized at 121°C for 20 minutes. All the cultures were maintained under a 16 and 8 h light and darkness, respectively, at 23 ± 2°C.
We chose to include activated charcoal in the medium as it enhances the growth of Phalaenopsis, as suggested by Ernst R (1975). The beneficial effects of charcoal on orchid seedlings or tissue culture-derived plantlets could be attributed to improved aeration. Another potential explanation is that charcoal absorbs ethylene and phenolic inhibitors known to impede growth and development.
Following a 4-week culture period, seedlings, measuring 0.5 cm in length with 1-2 leaves and

one or two roots, derived from the in vitro protocorm-like bodies (PLBs), were transferred to fresh media. Monthly replating was conducted to sustain the development rate of the plantlets. Each month, observations were recorded, including the count and length of leaves and roots, the dimensions of the plantlet, and the maximum width of leaves.

3. ***In Vitro* Multiplication**

During the multiplication stage, a variety of explants, including protocorm-like bodies (PLBs), leaves, stems, and root tips obtained from 6-month-old plants cultivated in vitro, were transferred to three different culture media under varying light conditions—both in darkness and in a 16/8 photoperiod. The first media selected was the most succesfull from the previous stage, (1) the ½ MS with MLG vitamins, microelements and iron at full strength, it did not contain growth regulators, it was used as control, while the other two had the same medium containing also

(2) 2 mg/l 6-Benzylaminopurine (BAP) + 0.5 mg/l 1-Naphthaleneacetic acid (NAA) + 100 mg/l Poly(vinylpyrrolidone) (PVP) + 1 g/l Activated Charcoal (AC), respectivly

(3) 2.5 mg/l BAP + 0.75 mg/l NAA + 100 mg/l PVP + 1 g/l AC.

All media were supplemented with 30 g·L-1 sucrose and solidified with 7 g·L-1 agar powder. pH was adjusted to 5.75 with 0.1M NaOH or HCl. Media were sterilized at 121°C for 20 minutes. Half of the cultures were maintained under a 16 and 8 h light and darkness, respect-tively and the other half were kept in darkness for the first 2 weeks/ when showing signs for the first leaf, both treatments at 23 ± 2°C.

Throughout micropropagation, the release of phenolic substances is a common occurrence, often negatively impacting the response of explants. Browning or blackening of cultured explants occurs only after the tissues are wounded, initiating the oxidation of phenolic substances regulated by polyphenol oxidase. The addition of activated charcoal (AC) mitigates the release of phenolic exudates. The positive effects of AC may stem from its ability to positively stimulate various developmental processes and absorb phenolic compounds, as suggested by Dipika Sarmah in 2017. The inclusion of Poly(vinylpyrrolidone) (PVP) in the medium also diminishes phenolic exudates. PVP serves a crucial role in plant tissue culture by inactivating or binding polyphenols and alkaloids that emanate from explants.

When choosing the explants, various factors were taken into account. Protocorm-like bodies (PLBs) intended for multiplication should exhibit normal size and color, and they should not surpass 2 months in age. Older, stagnant, or deteriorated PLBs tend to yield abnormal plants with stunted growth, both in vitro and *in vivo*. Additionally, small or yellow-colored protocorms are deemed unfit and are consequently discarded.

In thin-leaf culture, it is imperative to choose young leaves, specifically the most recently formed leaf. The segment for culture is transversely cut from the basal portion of this leaf, with segments measuring 1 mm. These segments are then positioned in the culture medium with the cut portion facing down and the adaxial side facing up.

For root-tip culture, only the tips of the roots, measuring under 0.5 cm, should be utilized. These root tips are placed in the culture medium with the cut portion facing down.

RESULTS AND DISCUSSIONS

1. Plant Material, Surface Sterilization of Capsule, *In Vitro* Asymbiotic Germination

The surface sterilization process was effective, enabling the seeds to germinate in the tested medium with a germination rate of 87.3%.

Phalaenopsis hybrid seed germination commenced with embryo swelling approximately three weeks after sowing. By the sixth week, embryos were released from the testa, transforming into rounded, yellow protocorms. At this stage, a shoot apex became visible at one side of the protocorm (see Figure 2). The protocorm progressed into an elongated shape, transitioning to a green color. Subsequently, absorbing hair developed, and the first and second leaves formed in succession. Roots emerged from the seedlings approximately nine weeks after sowing. The regular replating of protocorm-like bodies (PLBs) derived from seeds holds significant importance in enhancing their survival rate.

In the future we want to evaluate if the introduction of plant hormones and/or organic compounds can improve the success rate of the germination.

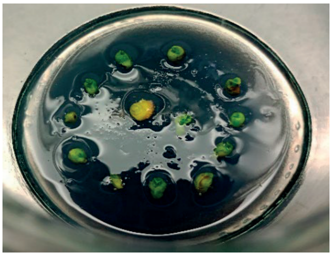

Figure 2. PLBs 7 weeks after sowing. Leaves forming, color changing from yellow to green

2. ***In Vitro* Protocorm-like Bodies (PLB) Development on Different Media**

Following the asymbiotic seed germination stage, the protocorm-like bodies (PLBs) were replated, and their development was assessed on four distinct media without plant growth regulators, as detailed in the Materials and Methods section 2.

The plantlets underwent relocation to fresh media every 30 days. Conclusions were drawn from measurements and observations made over the course of 4 months of cultivation (refer to Figure 3).

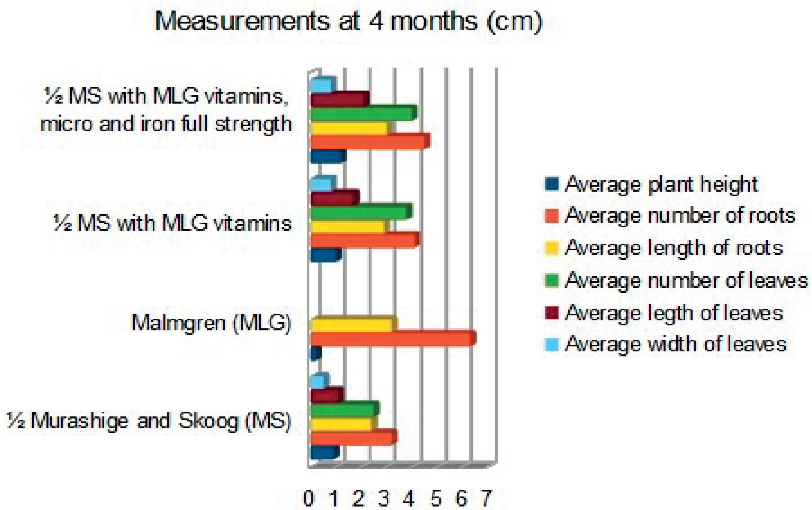

Figure 3. Measurements taken 4 months after starting the experiment

The 1/2 MS medium with MLG vitamins and microelements, including iron at a normal concentration (Option 4), demonstrated the highest suitability by providing the maximum number of leaves, along with optimal root and leaf length, offering overall favorable conditions for the development of protocorm-like bodies (PLBs) in general (see Figure 4).

The 1/2 MS medium with MLG vitamins (Option 3) also supported plant growth, although measurements revealed inferior results compared to medium Option 4.

While the MLG medium (Option 2) produced the longest roots, it was deemed less suitable as it only induced root regeneration and restricted stem development. This medium could be employed for rooting or as a source for root-tip culture (refer to Figure 5).

Maintaining the 1/2 MS medium (Option 1) beyond the germination stage impeded the growth of plantlets, with measurements after 4 months indicating poor results. After this stage, the plants exhibited an increased demand for vitamins and iron, and failure to meet this demand led over time to halted development or dwarfism.

Figure 4. Six month old plants grown on 1/2 MS with MLG vitamins and microelements, including iron at normal concentration

Figure 5. MLG medium was the most unsuitable, as it induced only regeneration of roots and restricted the development of stem

3. ***In Vitro* Multiplication**

During the multiplication stage, we assessed three media, as detailed in the Materials and Methods section 3. The evaluation included four types of explants: protocorm-like bodies (PLBs), leaf-thin sections, root tips, and stems (6 months old), conducted under both light and darkness conditions (see Figure 6).

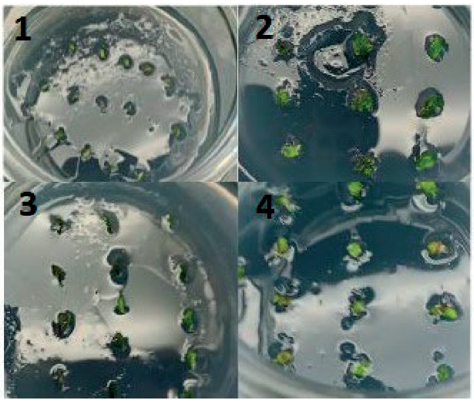

Figure 6. Explants at the time of initiating multiplication
1 - Root-tip culture; 2 - Stem culture;
3 - Thin-leaf culture; 4 - PLB culture

Phalaenopsis is recognized for its production of phenolic compounds. To mitigate the associated risks, we employed explants sourced from tissue culture, known for lower phenolic production. Additional measures, including Polyvinylpyrrolidone (PVP), Activated Charcoal (AC), initial darkness conditions for the first 2 weeks or until the emergence of the first leaf, and regular replating to fresh media, collectively contributed to the success of the experiment. These strategies effectively controlled the production of phenolic compounds, a common challenge in the multiplication phase. We recommend the use of PVP and AC specifically in root-tip culture and leaf-thin section culture.

In leaf and root-tip cultures, the presence of hormones is essential for eliciting an explant response. In the absence of hormones in the control media, regeneration of leaves and root-tips did not initiate. On the contrary, for all other media with hormones, regeneration commenced after 9 days, irrespective of light or dark conditions. Stems and protocorm-like bodies (PLBs) exhibited the fastest response in both conditions.

These conclusions are drawn from observing the explants for a duration of 3 weeks, and it's important to note that the experiment is still ongoing.

Protocorm-like bodies (PLBs) exhibited optimal development in the dark, particularly on medium version (2). Stems displayed varied responses based on light conditions; those kept in the dark developed into PLBs, while those in the light formed stems with new leaves. The most favorable overall development occurred in stems in the dark, specifically on medium version (3).

Leaf segments and root tips demonstrated accelerated development in the dark, especially on medium version (3). During this stage, explants exhibited a superior response in darkness, and we recommend this light regime for its efficiency and cost-effectiveness. However, extending the period of darkness beyond the initial phase, after eliciting a response, is known to inhibit their development.

CONCLUSIONS

Phalaenopsis orchids stand out as one of the most favored orchid varieties, valued for their ease of cultivation in home environments and their significant economic importance as sought-after ornamental cut flowers. The swift and uncomplicated propagation through seed-derived methods has proven to be an efficient approach, providing hybridizers with extensive selection possibilities for establishing future valuable hybrids. This approach is made feasible through the adoption of tissue culture techniques.

Tissue culture methods, in comparison to traditional breeding, require less time for the development and maintenance of varietal purity. This allows for the creation of new varieties with desirable traits such as compact growth habits, variegated foliage, and peloric flowers, enhancing the potential for orchid improvement.

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IN VITRO EVALUATION OF SALT AND DROUGHT STRESS TOLERANCE IN *HYPERICUM CALYCINUM* L.

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Abstract

Hypericum calycinum L. is a species of the genus Hypericum with pharmaceutical, ornamental, and landscape potential. To assess the salt and drought tolerance stress, in vitro cultures grown on media supplemented with different concentrations of NaCl (0; 10; 30; 50; 100 mM) and PEG 6000 (0; 10; 20; 30; 50 g/l) were used. After six weeks of in vitro culture under salinity and drought stress conditions, the following parameters were evaluated: viability, number of shoots, shoot length, rooting percentage, number of roots, root length, fresh weight, dry weight, water content, stress tolerance index (STI), and McKinney index (MKI). The viability of initial explants was higher under low salt stress (100% under 10 mM NaCl) compared to low drought stress (80% under 10 g/l PEG 6000). Drought stress caused a decrease in shoot height under all concentrations of PEG 6000, while the longest shoots (4.38 ± 0.22 cm) were obtained on the culture medium supplemented with 10 mM NaCl. The rooting percentage was 0% using concentrations of 30, 50, and 100 mM NaCl and 20, 30, and 50 g/l PEG. Our results showed that H. calycinum had a higher sensitivity to drought stress compared to salt stress.

***Key words**: abiotic stress, drought, in vitro, PEG 6000, salinity.*

INTRODUCTION

Hypericum species are found as wild or cultivated plants on all continents, except Antarctica and their habitus varies from herbaceous plants to trees, belonging to the genus *Hypericum* (Crockett & Robson, 2011; Ma et al., 2021; Danova et al., 2022). Hypericum species are known especially for their medicinal value (Azeez et al., 2017). Some species are also used for ornamental purposes due to their golden yellow flowers, ornamental fruits, and foliage shape (Ji et al., 2017).

Hypericum calycinum L., like other species of this genus, is used for medicinal purposes, especially to eliminate muscle spasms and for the treatment of asthma (Özkan & Mat, 2013). As an evergreen species that blooms from May to August and covers the ground well due to its growth form, it has significant ornamental and landscape potential. *H. calycinum* is used in some areas for landscape design, but its potential is much greater (Yücel et al., 2020). To expand the use of *H. calycinum* in landscape design to other areas, it's important to understand its behavior under drought and saline stress conditions.

In vitro cultures are frequently used to investigate plant responses to various abiotic stresses because they eliminate the influence of environmental factors that are difficult to control in field conditions. Moreover, they enable the rapid evaluation of a large number of species, cultivars, and clones in a small space and without soil impact in experimental fields (Hundare et al., 2022; Vuksanović et al., 2022; Garramone et al., 2023; Wijerathna-Yapa & Hiti-Bandaralage, 2023).

In previous research, saline stress conditions were induced by adding NaCl to culture media (Azzam et al., 2021; Ezzat et al., 2021; Hannachi et al., 2021; Sané et al., 2021; Alenezi et al., 2022; Jalili et al., 2022; Makkar et al., 2022; Asthana et al., 2023; Granata et al., 2023). To induce drought stress, PEG 6000 was frequently used (Mansinhos et al., 2022; Molnar et al., 2023; Aziz et al., 2023; Beyaz, 2023; Hanif et al., 2023; Mehmandar et al., 2023; Seyed Hajizadeh et al., 2023).

In vitro cultures in media supplemented with different concentrations of NaCl and PEG 6000 have been used to study the response to salt stress and drought stress for many species: *Solanum tuberosum* (Ezzat et al., 2021), *Solanum melongena* (Hannachi et al., 2021), *Solanum lycopersicum* (Sané et al., 2021), *Salvia officinalis* (Alenezi et al., 2022), *Medicago sativa* (Jalili et al., 2022), *Thymus lotocephalus* (Mansinhos et al., 2022), *Vaccinium corymbosum* (Molnar et al., 2023), *Sapindus trifoliatus* (Asthana et al., 2023), *Musa acuminata* (Aziz et al., 2023), *Lotus corniculatus* (Beyaz, 2023), *Ficus carica* (Granata et al., 2023), *Cucumis melo* (Mehmandar et al., 2023), *Rosa damascena* (Seyed Hajizadeh et al., 2023).

This research aimed to evaluate the difference between the response of *H. calycinum* to salinity and drought stress using *in vitro* cultures in media supplemented with different NaCl and PEG 6000 concentrations.

MATERIALS AND METHODS

***In vitro* treatment of salt and drought stress using NaCl and PEG 6000**

To study the effect of salinity and drought on *H. calycinum*, six-week-old *in vitro* cultures grown on Murashige and Skoog, 1962 (MS) culture medium without plant growth regulators (PGRs) were used.

To induce salinity and drought stress, the MS culture medium without PGRs was supplemented with 0; 10; 30; 50; 100 mM NaCl and 0; 10; 20; 30; 50 g/l PEG 6000, respectively. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl before adding the gelling agent. To solidify the medium 5 g/l (w/v) Plant agar was used and the medium was autoclaved for 20 minutes at 121°C. The vessels used consisted of 720 ml (v/v) culture jars (9 cm in diameter and 13.5 cm high) with transparent polypropylene caps.

100 ml (v/v) culture medium was dispensed into each jar and 15 explants were inoculated, each being 1.5-2 cm long.

The *in vitro* cultures were incubated in the growth room at a 16 h photoperiod, 32.4 µmol $m^{-2}s^{-1}$ light intensity (cool white fluorescent light, Philips), and temperature of 23 ± 3°C.

All chemicals were obtained from Duchefa Biochemie B.V, The Netherlands.

Growth Parameters

The growth response to salinity and drought stress was determined after six weeks of in vitro culture conditions, for the following parameters:

- number of shoots - the average number of shoots produced per initial inoculum;
- number of roots - the average number of roots produced per plantlet;
- shoot length (cm) - the average length of shoots produced per initial inoculum;
- root length (cm) - the average length of the roots produced per plantlet;
- mean fresh weight (FW) of shoots per explant (mg): 15 explants for each treatment were weighed immediately after the material was removed from the *in vitro* culture medium;
- mean dry weight (mg): the material was dried for four days at 25°C and re-weighted (DW);
- average water content (WC): based on FW and DW, the WC percentage was calculated using the formula (Mazurek et al., 2021):

$$WC\ (\%) = [(\text{Fresh Weight} - \text{Dry Weight}) / \text{Fresh Weight}] * 100$$

Calculation of tolerance indices

Stress Tolerance Index (STI). Based on the collected data, the response to salinity and drought stress was evaluated using the stress tolerance index (STI). The STI for salinity stress was calculated as the ratio between the performance of each parameter at 10, 30, 50, and 100 mM NaCl and the performance traits at 0 mM NaCl. The STI for drought stress was calculated as the ratio between the performance of each parameter at 10, 20, 30, and 50 g/l PEG and the performance of the trait at 0 g/l PEG, according to the following formula (Zaki & Yokoi, 2016):

$$STI = Ts/Tp$$

where:

- Ts is the trait of genotype under stress treatments;

- Tp is the trait of genotype under normal conditions.

High STI values indicate tolerance to salinity or drought stress.

McKinney index (MKI). Evaluation of the chloroses and/or necroses induced by salinity and drought stress was performed by ranking each shoot into ten classes (Table 1) using a modified McKinney Index (MKI) (Di Cori et al., 2013) according to the following formula:

$$MKI = \Sigma(ni \times i)/N$$

where:

- ni is the number of shoots assigned to the class;
- i is the numeric value of the class;
- N is the total number of examined shoots at each salt and PEG 6000 concentration.

Data are the average value of 30 plants (two jars) for each treatment.

Table 1. The numerical value of the ten classes corresponding to the McKinney index index (MKI)

Class	Symptoms
0	No injury
1	Brownish of basal part of the stem
2	Up to 20% chlorosis
3	From 20 to 50% chlorosis
4	Up to 10% brownish on stem or over 50% chlorosis on whole plant
5	From 10 to 30% brownish on stem
6	Necroses on apical leaves
7	From 30 to 50% necroses on stem
8	Growth reduced or 50% necroses on stem
9	Growth inhibited or necroses on whole stem
10	Necroses on whole plant

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) and post hoc testing for the ANOVAs was performed using Tukey's honestly significant difference test (Tukey's test) using a $P<0.05$ significance level to determine the statistically significant differences between the means. Values shown (in text and figures) are means ± SE (standard error).

RESULTS AND DISCUSSIONS

After six weeks of exposure to stress with NaCl and PEG 6000, *H. calycinum* exhibited different responses to the two stress factors (Figure 1).

The viability of initial explants was higher under salinity stress compared to drought stress. Under the lowest concentration of NaCl (10 mM), the viability was 100%, the same as on the culture medium without stress, while under the lowest concentration of PEG (10 g/l) was 80% (Table 2).

The length of the shoots was significantly influenced by the concentration of PEG in the growth medium and decreased significantly under all concentrations (Figure 2). The shoot length decreased 2.8 times under the concentration of 10 g/l PEG, while under the highest concentration of PEG (50 g/l), it decreased 3.8 times compared with the unstressed culture medium.

Table 2. Viability of initial explants under salinity and drought stress

Treatment	Viability of inocula (%)	Rooting percentage
Control	100.00 %	80.00 %
10 mM NaCl	100.00 %	60.00 %
30 mM NaCl	73.33 %	0.00 %
50 mM NaCl	73.33 %	0.00 %
100 mM NaCl	46.67 %	0.00 %
10 g/l PEG	80.00 %	13.33 %
20 g/l PEG	80.00 %	0.00 %
30 g/l PEG	66.67 %	0.00 %
50 g/l PEG	60.00 %	0.00 %

On the other hand, under the lowest NaCl stress (10 mM), the shoots exhibited a longer length (4.38 ± 0.22 cm) compared to the non-stressed culture medium (3.94 ± 0.28 cm), suggesting that *H. calycinum* may be considered a mildly halophytic species.

However, under the highest concentration of NaCl (100 mM), shoot growth was more strongly inhibited (0.39 ± 0.22 cm) compared to the highest concentration of PEG (50 g/l), which resulted in shoots with a length of 1.01 ± 0.07 cm. This result is not unusual, a positive effect of low NaCl concentrations has been reported in other species as well. For example, Zayova et al. (2017) demonstrated that under 50 mM NaCl, plant height and root length were greater compared to the unstressed control under in vitro growth conditions of *Solanum melongena*. Also, in comparison with *H. calycinum*, the length of the shoots of *Viola odorata*, a medicinal and ornamental species, decreased with the increase of both PEG 6000 and NaCl concentrations (Darvishani et al., 2020).

As shown in Figure 3, the number of regenerated shoots was strongly influenced, either positively or negatively, by the two stress factors. Compared to the control, the number of

regenerated shoots decreased under all NaCl concentrations, from 2.22 ± 0.17 (control) to 1.14 ± 0.07 (100 mM NaCl). In contrast, the number of regenerated shoots increased with the increase in PEG concentration. Under the concentration of 50 g/l PEG, the number of regenerated shoots was 3.25, 1.5 times higher than the control. The increase in the proliferation rate in the presence of PEG 6000 was also reported in the case of *Vaccinium. corymbosum* grown *in vitro* under different concentrations of PEG (Molnar et al., 2022).
On the other hand, in strawberries grown in vitro under drought stress, the number of regenerated shoots decreased with the increase in PEG concentration (Clapa & Harta, 2021).
The detrimental effect of these stresses was more pronounced regarding the roots, as indicated in Table 2 and observed in Figure 2. The rooted plantlets were obtained only under low concentrations of NaCl (10 mM) and PEG (10 g/l). When grown on the unstressed culture medium, the plants rooted 80% and under 10 mM NaCl, this percentage decreased to 60%, and under 10 g/l PEG, it plummeted to 13.33%. The number of roots was significantly reduced under 10 g/l PEG (0.22) but remained unaffected under 10 mM NaCl (1.70) compared to the control (1.69). These results highlight the more pronounced detrimental effect of drought stress on root development. The presence of NaCl and PEG in the culture media affected the percentage of rooting, and the number and length of roots in *Stevia rebaudiana* as well (Magangana et al., 2021). The fresh weights and dry weights of shoots decreased under all concentrations of PEG and NaCl, with the most significant reduction observed under 100 mM NaCl (42.60 ± 3.25 mg). The highest shoot fresh weight was recorded in control plants (109.46 ± 13.25 mg) (Figure 4).
Salinity tolerance and drought tolerance are expressed by the stress tolerance index in Table 3. The general trend was a decrease in ITS with increasing salinity and drought levels except for the number of proliferated shoots under PEG concentrations. In this case, the drought tolerance index increased with the increase in the concentration of PEG, confirming the fact that the presence of PEG in the culture medium leads to an increase in the number of proliferated shoots of *H. calycinum*.

To evaluate the degree of chlorosis and necrosis of *H. calycinum* shoots under salinity and drought stress, the McKinney index (MKI) was used (Table 3). No chlorotic leaves or brown shoots were observed in *H. calycinum* plants exposed to 10 mM NaCl, and the MKI value was 0. In contrast, plants exposed to 100 mM NaCl were the most necrotic, with MKI=30.80 (Table 4). MKI shows that plants exposed to drought stress had necrosis under all concentrations of PEG, the most affected being under 50g/l PEG (MKI = 16.80).

Table 3. Stress tolerance index (STI) for *H. calycinum* maintained for four weeks in culture media with different concentrations of NaCl and PEG 6000

Treatment	No of shoots	No of roots	Shoot length (cm)	Roots length (cm)
10 mM NaCl	0.66	1.00	1.11	0.77
30 mM NaCl	0.57	0.00	0.58	0.00
50 mM NaCl	0.61	0.00	0.66	0.00
100 mM NaCl	0.51	0.00	0.10	0.00
10 g/l PEG	1.07	0.13	0.35	0.24
20 g/l PEG	1.41	0.00	0.30	0.00
30 g/l PEG	1.35	0.00	0.28	0.00
50 g/l PEG	1.46	0.00	0.26	0.00

Table 4. McKinney index (MKI) for *H. calycinum* maintained for four weeks in culture media with different concentrations of NaCl and PEG 6000

Treatment	MKI
Control	0.00
10 mM NaCl	0.00
30 mM NaCl	0.83
50 mM NaCl	3.33
100 mM NaCl	30.80
10 g/l PEG	0.20
20 g/l PEG	0.83
30 g/l PEG	8.67
50 g/l PEG	16.80

Salinity and drought stress induced in this study by supplementing the culture media with different concentrations of NaCl and PEG showed that *H. calycinum* has a higher sensitivity to drought stress compared to salinity stress (Figure 5).
Our results showed that high salinity and drought caused a reduction in the growth of shoots and roots of *H. calycinum* grown in vitro and confirmed that the strategy of stressed plants is to slow down their growth (Hossain et al., 2007).

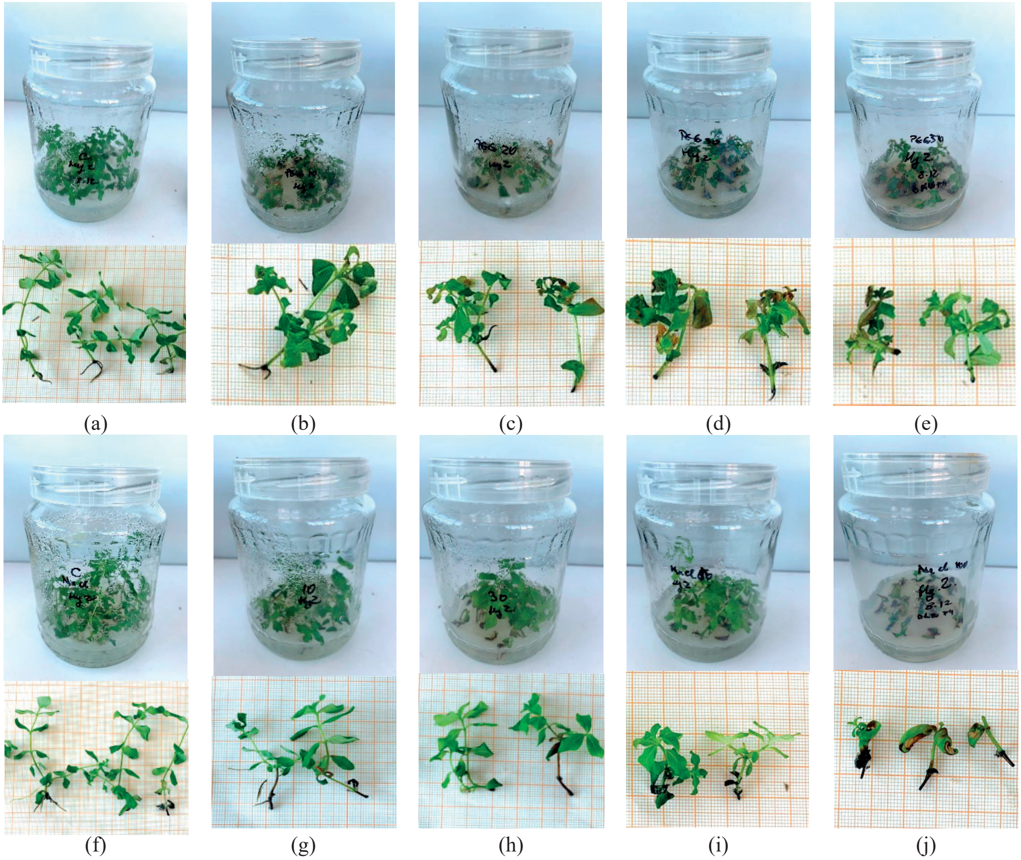

Figure 1. Effect of different concentrations of PEG 6000 and NaCl on in vitro culture of *H. calycinum*: control (a, f), 10 g/l PEG 6000 (b), 20 g/l PEG 6000 (c), 30 g/l PEG 6000 (d), 50 g/l PEG 6000 (e), 10 mM NaCl (g), 30 mM NaCl (h), 50 mM NaCl (i) and 100 mM NaCl (j)

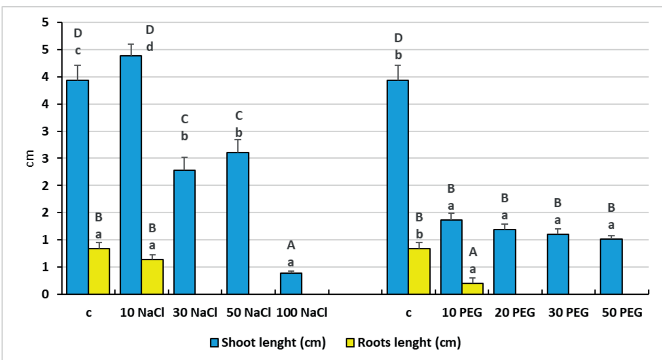

Figure 2. The length of shoots and roots of *H. calycinum* under salinity stress (0, 10, 30, 50, and 100 mM NaCl) and drought stress (0, 10, 20, 30, and 50 g/l PEG 6000). Different lowercase letters indicate significant differences among the same treatments and different capital letters indicate significant differences among all treatments according to Tukey's HSD test ($P \leq 0.05$). Error bars indicate mean ± SE

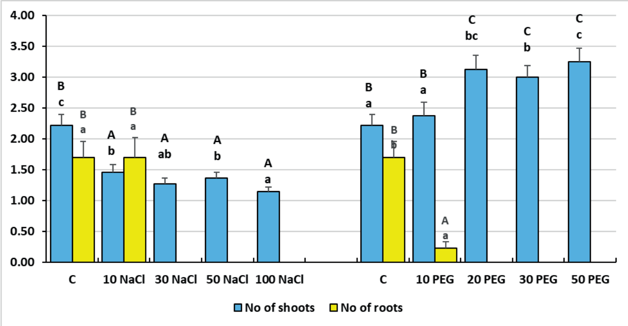

Figure 3. Number of shoots and number of roots per initial inoculum of *H. calycinum*. For inducing the salinity stress in culture media were added 0, 10, 30, 50, and 100 mM NaCl and for inducing drought stress were added 0, 10, 20, 30, and 50 g/l PEG 6000. Different lowercase letters indicate significant differences among the same treatments and different capital letters indicate significant differences among all treatments according to Tukey's HSD test (P ≤ 0.05). Error bars indicate mean ± SE

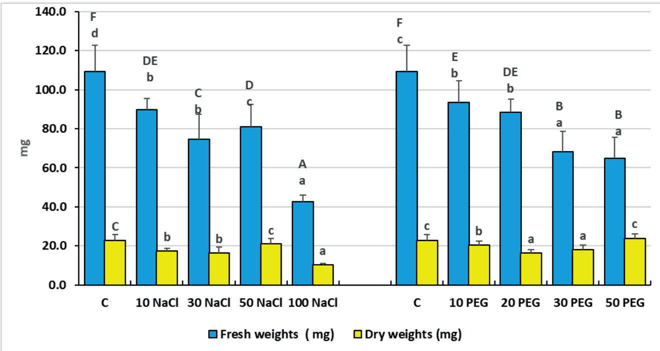

Figure 4. Fresh weights (mg) and dry weights (mg) of the shoots of *H. calycinum* under salinity stress (0, 10, 30, 50, and 100 mM NaCl) and drought stress (0, 10, 20, 30, and 50 g/l PEG 6000). Different lowercase letters indicate significant differences among the same treatments and different capital letters indicate significant differences among all treatments according to Tukey's HSD test (P ≤ 0.05). Error bars indicate mean ± SE

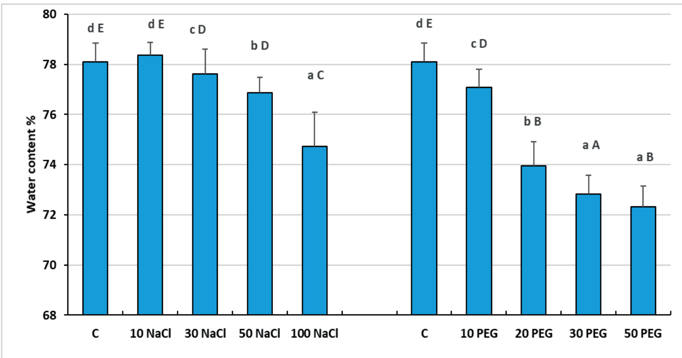

Figure 5. Water content of the shoots of *H. calycinum* under salinity stress (0, 10, 30, 50, and 100 mM NaCl) and drought stress (0, 10, 20, 30, and 50 g/l PEG 6000). Different lowercase letters indicate significant differences among the same treatments and different capital letters indicate significant differences among all treatments according to Tukey's HSD test (P ≤ 0.05). Error bars indicate mean ± SE

CONCLUSIONS

In conclusion, our results showed that *H. calycinum* exhibits greater sensitivity to drought stress compared to salt stress. Specifically, the height of the shoots was higher at low salt concentrations (10 mM NaCl) compared to those obtained in the absence of stress factors. This suggests that *H. calycinum* may be suitable for landscaping in areas with lands with a low degree of salinity. To validate this finding, field research should be extended.

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A REDEVELOPMENT SOLUTION FOR THE "ALEXANDRU SAHIA" PARK IN RĂDĂUȚI

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Abstract

This study investigates the rehabilitation of a green area found in the city of Rădăuți, Suceava. Since the '60s, the "Alexandru Sahia" Park has been a suitable oasis for its inhabitants, for socializing and relaxation, especially during hot summer days. Considering its current state of deterioration, mainly due to decay of its elements and poor maintenance works, the park no longer fully meets the needs of the community. The proposed solution aims to fulfil the functional, environmental, and aesthetic requirements so that each area can reach its optimal potential from all perspectives and put at the community's disposal a relaxation and recreation oasis, where people could rest or have fun, socialize, and benefit from a higher quality of life. The solution meets both the needs of the citizens living near the park and of the students passing through the park on their way to the three nearby educational institutions.

***Key words**: landscaped green areas, redevelopment solution, urban park.*

INTRODUCTION

The evolution, dynamics, and development of cities define today's civilization (Balan-Ionescu (Popescu) and Toma, 2023). Considering this development, urban parks have become one of the key elements in cities, acquiring a growing importance in the lives of city residents (Sadeghian and Vardanyan*,* 2013) and bringing numerous environmental, physical, psychological, and social benefits. Urban parks are highly important for maintaining and improving physical well-being and health. Their natural elements contribute to lower daily stress, better mood, higher degree of relaxation, and lower levels of mental disorders. (Annerstedt et al., 2012). Parks contribute to achieving social inclusion by creating spaces for social interaction, having a much greater potential compared to other urban facilities due to easy and unlimited access. People need to meet helping a community be able to develop its social ties. Plus, people through interaction actively engage in society and develop feelings of acceptance, set and strengthen relationships through communication and participation in joint activities. (Konijnendijk et al., 2013). Through their social function, parks bring both educational and scientific benefits. Users develop their civic sense, self-respect, and respect for the city through cooperation, personal development, and desire for collective well-being when getting together in landscaped areas (Dascălu D.M, 2016). It is a well-known fact that recreation is closely linked to mental and physical health. A rested body is much more capable of recovering its physical and intellectual strength. During the summer, when temperatures exceed bearable levels, urban parks become places of coolness and relaxation and provide opportunities for active or passive rest. Plus, children develop in their early years through play. It is natural for children to love parks and playgrounds. Studies show that time spent outdoors helps children develop their motor and cognitive skills more effectively and stay more active physically (Li et al., 2022).

Vegetation, parks, private gardens, street alignments, green roofs and facades, as well as water areas, are just a few examples of urban green infrastructure counteracting the negative effects of urban overcrowding. (Aigbokhan et al., 2023; Cojocariu et al., 2022). Along with the earlier mentioned benefits, green areas add a decorative value to a territory that can be associated with the satisfaction a person might have regarding the existing vegetation and its artistic presentation. Therefore, parks can also bring aesthetic benefits meeting the need for beauty and contributing to lowering the aridity of densely built areas (Dascălu and Cojocariu, 2016).

This study aims to propose a landscape rehabilitation solution for the "Alexandru Sahia" Park in the city of Rădăuți, Suceava County. Its main aim is to enhance the quality of this green space through architectural and landscaping developments of all functional areas of the park.

Such underused areas may be found in any city. It usually happens whether due to unsustainable exploitation or due to a wrong approach. Therefore, rehabilitation solutions are made for areas with high potential. The two examples in this sense are the Metropolitan Park in Târgoviște (Pașcu et al., 2021) and the Dendrological Park in Buhusi, Bacau County (Pașcu et al., 2022).

Landscape rehabilitation aims to preserve the current functions of the park and add new elements needed to create a modern urban framework meeting the current needs of the city's residents in the best possible way. The proposal is targeted not only at those living near the park but also at students passing through the park on their way to the three nearby educational institutions: General School no. 5; Technical College of Rădăuți and Eudoxiu Hurmuzachi National College (Figure 1).

Figure 1. City plan of the park and the three educational units (67 - "Alexandru Sahia" Park; 19 - General School no. 5; 20 - Rădăuți Technical College; 22 - Eudoxiu Hurmuzachi National College)

MATERIALS AND METHODS

To carry out the study, we have reviewed the literature in the field and studied the archival resources on the park's history. We have used the following methods investigations, on-site analysis, review, and interpretation of the current literature.

After having done the on-site analysis and the literature review, we have developed a concept for restoring this green space. To reach the aim, the following objectives were taken into account:

- Refurbishment and design of new pathways ensuring access to all areas of interest;
- Refurbishment of the building, the island, and the basin;
- Design of a new playground area with elements divided by age groups;
- Set up relaxation and study areas for students;
- Proper furnishing of all areas;
- Restoration of the existing vegetation and removing unesthetic specimens or those that is in danger of falling.
- Proposals for new plant compositions.

RESULTS AND DISCUSSIONS

The level of attractiveness of green spaces is primarily determined by their ability to meet the needs for urban comfort and quality of life, in general (Cojocariu et al., 2023). Currently, various forms of urban green spaces are the main elements of the built environment affecting directly the quality of life (Istrate et al., 2023).

The "Alexandru Sahia" Park is just a few steps away from the House of Culture and 500 meters away from the historic city centre of Rădăuți. Its surface is approximately 11.300 m² (Figure 2).

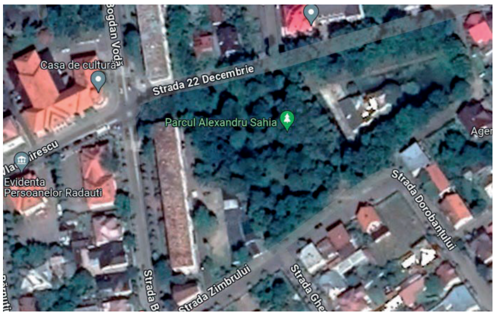

Figure 2. Aerial view of the "Alexandru Sahia" Park in Rădăuți (Google Earth)

The park was built at the beginning of the fifties, being back then a landmark of the city of Rădăuți. A postal card dating back to 1953

(Figure 3), shows the image of the island in the park of the time.

Figure 3. A postal card - The picture of the island in the "Alexandru Sahia" Park
(Photo 1953, Ion Petcu)
(https://cartipostale.cimec.ro/Detaliu.php?id=11397&criteriu=pat)

The park called by the people "On the Lake" was seen by the locals as a beautiful spot with many weeping willows around the lake and in the middle of the island. The boat rides on the lake were a common entertainment activity back then. Another postal card from the eighties shows the charm of this place created in a city in the province (Figure 4).

Figure 4. Postal card – View from the park (1980)
https://www.hippostcard.com/listing/postcard-romania-radauti-vedere-din-parc-lac-pord-natura/27590127

The existing buildings in the park today were built later, losing quickly their usefulness. Their questionable architectural quality and the fact that there was no unified approach to the whole area are the two possible causes of their abandonment. In recent decades, the park has not been a priority for the local authorities. Without proper maintenance, its elements, both built and natural, have deteriorated significantly. There have been attempts to revitalise the area, but these have not been backed up by a coherent and integrated vision. The idea for a redevelopment proposal came from a desire to restore the park to its former beauty and usefulness.

The analysed area comprises a series of functional areas, such as green spaces, promenade areas, a playground, an area of fitness bars, a lake, and an island located in the center. The site analysis underlines the degree of deterioration of the lake basin, pathways, furniture, and the state of aging or unhealthy plant specimens. Also, destroyed or even missing railings surrounding the island and the lake, as well as some damaged parts of the playground may become a real danger for children. The built elements on the lake have been abandoned and the lack of maintenance enhance the sense of area's abandonment. There have been acts of vandalism, and in combination with litter and lack of park maintenance, the overall image is deplorable (Figure 5).

Therefore, measures are needed to repair these irregularities in the existing areas and propose new functional areas which would meet the residents' need for resting and socialising places. Plus, the building found on the island could provide a suitable space for setting up a café or a restaurant, which, together with the lake area, may contribute to the functional and aesthetic enhancement of the entire park.

The furniture is damaged, and dirty, its quality being poor and unsuitable for an urban area. Trash bins are insufficient or improperly positioned, litter is being thrown into the lake. The furniture in the children's playground area is destroyed, posing a real danger for children. The park lighting is not functional, insufficient for the park's needs, and severely damaged. The water in the lake is dirty, full of garbage, and could pose a risk to the health of the residents.

Figure 5. Images showing the current state of the park (original)

Also, the insular shore has a high risk of collapse as portions of it have already detached. The building on the island was abandoned, and its annexes serve no functional or aesthetic purpose. The functional areas in the park comprise an inadequately placed, aesthetically unpleasing, almost entirely destroyed playground and a recently arranged area of fitness bars, although being in a better condition, they are disconnected from the overall layout and seem to be unused due to the park's overall appearance. This area features a non-aesthetic enclosure bringing a sense of isolation from the rest of the park.

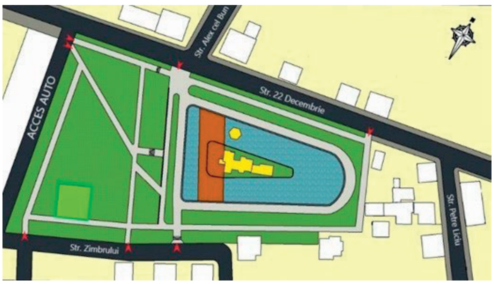

Figure 6. Current park plan

After identifying the actions and the elements requiring either changes or improvements, we proposed rearranging specific park areas and functions, and also for rehabilitating the existing ones. The layout of the pathways is shown on the plan describing the current situation (Figure 6). Many of these pathways provide direct access to the roadway, with the side path serving as a sidewalk for those wishing to cross the nearby 22 December Street. On the opposite side, there is no sidewalk, forcing pedestrians to walk on the lawn area when crossing Zimbrului Street.

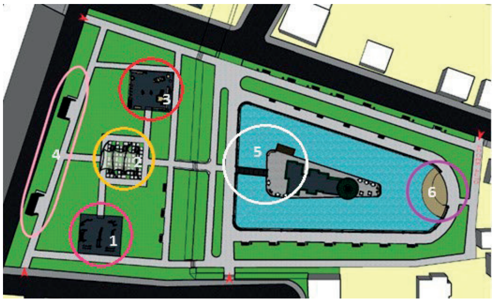

Figure 7. Proposed landscape redevelopment plan

The pathways inside the park have been partially redesigned so that it could ensure a good connection between the proposed functional areas. Plus, a sidewalk strip was proposed on Zimbrului Street. The island cannot be accessed as there is no access route to it. The island comprises some deck extensions, on which there had been previously arranged terraces that are currently in disarray. The existing fitness area (1) in the park has been linked with the rest of the proposed areas by removing the fencing around it and incorporating it into a compositional design. Also, there is a children's playground area (3), a study/dining area (2), and several spatial extensions for relaxation (4) (Figure 7).

The Lake

The park's main attraction point is the lake. "Water has the gift of adding charm to a landscape, especially when accompanied by ingenious landscaping, balanced architectural elements, and subtle plant arrangements". (Dascălu and Cojocariu, 2016).

Figure 8. Front view of the deck

"Water has the quality of drawing attention to a landscape, being one of its most interesting elements" Al-Suwaid et al, 2022). Therefore, the proposal aims to refurbish the lake and create an ambiance made of architectural elements and specific vegetation.

Also, the proposal suggests the creation of a habitat for lake-specific bird species, so a deck will be built in its vicinity (area 6 in Figure 7), where users will be able to interact with the birds or cool off near the water. Suitable vegetation is proposed for the lake area (Figure 8), typical for the lake water and its sides. There could be encountered such water-loving species as *Typha minima*, *Scirpus lacustris „*Albescens*"* and *Scirpus tabernaemontani „*Zebrinus*"*, *Juncus ensifolius* and *Phragmites karka* (Figure 9).

Figure 9. Side view of the deck

The island and the building

The refurbishment proposal contains the removal of the annex buildings on the site to create a suitable area for strolling around the lake. Moreover, a bridge (area 5 in Figure 7) is aimed to be built in order to connect the island to the rest of the park (Figure 10). The old railings were replaced with the new ones harmonising with the rest of the arrangement. A small pier was built, from where visitors can rent water bikes (Figure 11).

Figure 10. Bridge view

Figure 11. Pier detail deck

The building on the island will host a restaurant. The island's land could serve as a space for arranging terraces (Figure 12).

Figure 12. View from the restaurant terrace

For the decoration of the terraces, we suggested several planters with plant compositions, mainly containing floral species and decorative grasses.


a


b

Figure 13. a, b Plant compositions in planters (original)

For the first composition (Figure 13 a), the following species were used: *Hydrangea macrophylla* “Little Pink”, *Spiraea japonica* “Little Princess”, *Matteucia strupthioides, Calamagrostis acutiformis* “Karl Foerster”, *Koeleria glauca*, *Dianthus caryophyllus* “Pale Pink”, *Dichondra argentea* “Emerald Falls”. The second composition (Figure 13 b) was made by combining the following species: *Heuchera micrantha* “Palace Purple”, *Pennsietum alopecuriodes* “Bianco”, *Koeleria glauca, Dichondra repens, Hedera helix* “Variegata”, *Phlox subulata* “White Delight”.

Functional areas

Besides the walks that visitors can take inside the park, they also need other well-designed spaces that would meet their various needs. To meet the need for relaxation, socializing, and beauty, two functional areas were proposed inside the park, in addition to the existing fitness area, a playground, and a study or outdoor dining area. The fitness area was enhanced by adding seats (Figure 14), made of high-quality and water-resistant material blending visually with the other elements in the surrounding areas.

The current chain-link fence was replaced with a green fence of *Lonicera nitida* “Maigruen”, which provides a touch of privacy to the area, besides its decorative role. The foliage and flowers of the fence will contribute to the overall decor.

Figure 14. Fitness area (plan and view)

A playground for children was designed with facilities suitable for multiple age groups. In terms of design, it matches the overall concept. For the age group of 0-2 years, we chose a small tunnel equipped with a very short slide and a sandbox. Children aged 2 to 5 can enjoy spring rockers and a supported swing, while those aged 6 to 9 could climb ropes or use the interactive wall to invent games, slide down slides, or use the larger swings, due to their greater agility and strength compared to the previous age group. Figure 15 illustrates some of the proposed playground equipment. The playground surface will be made of protective material ensuring safety in case of falling.

In the relaxation area arranged for studying or dining (Figure 16), we suggested building a pergola providing protection against birds nesting in the nearby trees. Tables with seating were placed so that students could meet after classes and work on assignments together, while the elderly could play strategy games. This space serves a dual purpose as it can also serve as a relaxation area.

Figure 15. Playground equipment

Figure 16. Study area (plan and view)

The land surface in the study area was made more dynamic by a play of scree and vegetation. To shape visually the area, we suggested the perimeter vegetation clusters. We suggested decorative grass species such as *Pennisetum alopecuroides, Festuca rubra, Festuca glauca, Miscanthus sinensis, Stipa calamagrostis* “Allgau”, *Hakonechloa macra*, and the *Juniperus procumbens* “Nana” shrubs. The ornamental assortment in this area was enriched by *Lavandula angustifolia.* During the evening, the decorative lighting ensures good visibility and contributes to the decoration of the entire area by means of luminous spheres placed directly on the lawn.

Figure 17. Pergolas

Adjacent to the pathway on the western side of the park, we added two more generous spatial extensions with pergolas (Figure 17), adding an extra touch of elegance to the entire park. These also serve a dual purpose, providing a relaxing and also an intimate space, shielded from the traffic flow of the pathways. The pergolas were decorated with *Lonicera japonica* “Halliana”.

Furniture

Urban furniture should display several basic qualities: comfort, security, shelter, and design (Mexi A. and Tudora I., 2012). So, the furniture was replaced by benches following the same style and rearranged in such a way that the view was not obstructed by trees. The ambient lighting of the park was done with classical lanterns matching the rest of the elements. Also, the aim was to prevent light pollution and reduce unnecessary energy consumption.

Vegetation

Most of the initially present vegetation in the park was preserved, the trees including species of with species wild chestnut *(Aesculus hippocastanum)*, green ash *(Fraxinus pennsylvanica)*, hornbeam *(Carpinus betulus)*, silver birch *(Betula pendula),* Norway maple *(Acer platanoides)*, black locust *(Robinia pseudoacacia)*, walnut *(Juglans regia)*, oak *(Quercus robur)*, silver lime *(Tilia tomentosa)*, Norway spruce molid *(Picea abies),* and white willow *(Salix babylonica).*

In the case of willow trees showing wounds, if healing could still occur after cleaning and treatment, then they will be preserved, while the specimens at high risk of collapse will be eliminated.

A hedge of *Prunus laurocerasus* was suggested for the sides of the park on the 22nd of December and Zimbrului Streets as to provide protection and prevent accidental access from the road. So as not to completely block visibility towards the park, the fence will be maintained at a maximum height of 1.5 m.

To achieve special aesthetic effects, plant compositions were created that could be found in several areas of the park. As the tree canopies cover almost the entire planting area, we have selected semi-shade tolerant species.

The lawn is the background stressing out the elements of the park. Taking into account the lack of irrigation, moderate degree of shading, low frequency of mowing, and its use as an ornamental lawn with low traffic, we are suggesting the use of a seeding mixture of *Festuca rubra, Trycophylla*, *Festuca rubra* ssp. Comutata and *Lolium perenne* for the lawn replantation. On the planting area, the soil will be prepared and fertilized to ensure proper plant development.

All these aspects presented in the proposal for the redevelopment and rehabilitation of the “Alexandru Sahia” Park contribute to the creation of an urban environment preserving nature, fighting pollution, and meeting the outdoor recreational needs of the residents. The elements were chosen in line with the style of the entire development by applying the principle of unity in diversity. The aim was also to achieve a stylistic unity between all elements as to achieve a harmonious and balanced space that complies with the principle of aesthetic values. Another principle used in the redevelopment proposal was that of efficiency. Therefore, the most suitable uses of the park were first identified and then used to make reasonable proposals to give a new life to the studied location by exploiting to the fullest the qualities of the landscaped area.

CONCLUSIONS

The article presents a proposal for transforming the currently neglected and unused space of the “Alexandru Sahia” park into a functional, aesthetic, and pleasant park.

Research conducted globally on the benefits of parks in enhancing the quality of the surrounding ecosystem and human life has shown that both the environment and people suffer without parks.

People go to parks to relax, refresh themselves, and lower the anxiety of everyday life. Leisure activities in nature improve the mood, reduce stress, and increase the quality of life.

The set objectives led to the delivery of a satisfactory result from all perspectives. Functional, aesthetic, and technical aspects of the space were taken into consideration, and an environment meeting both practical and emotional needs of the park users.

The suggested proposal puts at the disposal of the community an oasis of relaxation and recreation, where residents can rest or have fun, socialize, and improve the quality of their lives in all possible ways.

Created functional and sustainable landscaped areas comprising a playground, a study area, a pier, a pergola area, and an island terrace provide to visitor's various leisure and recreation options.

The lake and the vegetation, the elements of the natural setting bring numerous physical and psychological benefits, and together with the other elements and furniture, create a new, modern, and sustainable ambiance environment.

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FROM AGRICULTURAL OASIS TO URBANIZATION: PATH OF OASIS GREEN INFRASTRUCTURES IN BISKRA, ALGERIA

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Abstract

This article explores the dynamic of oasis green infrastructure in Biskra, Algeria, highlighting the crucial role of palm groves in urban sustainability. By revisiting its traditional past and assessing its current impact on urban sustainability, the study highlights the importance of the oasis of Biskra as an essential connector between cultural heritage and contemporary challenges. Analytical methods encompass historical, geographical and environmental assessment approaches to trace the evolution of green infrastructure in the pre-colonial, colonial and post-independence periods. Indicators of surface area and types of green infrastructure were used to quantify changes. The results highlight the capacity of the palm grove to adapt while preserving its crucial role in providing social, economic and environmental services, underlining the environmental issues associated with its degradation. In conclusion, the Biskra palm grove emerges as an essential element of urban sustainability, illustrating the need to preserve this traditional ecosystem. Integrating sustainable practices into palm grove management can strengthen urban resilience and promote a harmonious balance between urbanization and ecological conservation.

***Key words**: oases, green infrastructure, palm groves, urban sustainability, biodiversity, cultural heritage, environmental assessment, environmental management.*

INTRODUCTION

Urban green infrastructure refers to the network of natural and semi-natural areas, such as parks, green roofs, street trees, rain gardens, and wetlands, designed and managed to provide ecological, social, and economic benefits in urban areas (Taylor Lovell and Taylor, 2013; Mell, 2008). It is a planning and design approach that recognizes the importance of incorporating nature into the built environment to enhance the quality of life for people and the environment (Cameron et al., 2012; Cilliers et al., 2013). Effective urban green infrastructure planning involves considering the needs and preferences of diverse stakeholders, as well as assessing the ecological and social value of green spaces, and integrating green infrastructure into urban design and development (Petrişor et al., 2021; 2022). The green infrastructure includes a variety of semi-natural areas along with green spaces intentionally created to provide diverse ecosystem services essential for human welfare and improving overall life quality (Gavrilidis et al., 2023). Additionally, green infrastructure answers to different economic, social, and environmental challenges (Tzoulas et al., 2007). More exactly, the urban green infrastructure plays a crucial role in mitigating the adverse impacts of urbanization, such as air and water pollution, heat island effect, and reduced biodiversity (McMahon, 2000; Gill et al., 2007). It can also provide numerous benefits, such as improving air and water quality, reducing energy use, enhancing mental and physical health, supporting local food

production, and increasing property values (Taylor Lovell and Taylor, 2013).
The ecological side of green infrastructure is crucial, as it emphasizes the need to preserve and promote natural ecosystems in urban areas. The term "green infrastructure" highlights the crucial role of nature in modern urban planning. These spaces are not isolated but are an integral part of any balanced and sustainable urban ecosystem (Walmsley, 2006).
The continuous growth of urban areas, at the expense of natural areas, poses an imminent threat to urban ecosystems. Several studies have highlighted the negative consequences of habitat fragmentation on biodiversity, leading to a concerning decrease in biological diversity and a significant reduction in the quality and amount of essential ecosystem services due to urban sprawl (Petrişor et al., 2021; 2022).
The benefits of ecosystem services highlight their importance even more in arid environments (Hadagha et al., 2018). In Biskra, and especially after the independence of Algeria in 1962, the loss of palm grove due to urban sprawl has particularly affected the palm groves which play an essential role as reservoirs of biodiversity, drivers of agricultural economy, and climate regulators for the oasis city. Urban sprawl was a consequence of derogatory planning, more intense after the independence (Hamma and Petrişor, 2018). Although palm trees are an important natural resource, they have suffered the negative consequences of rapid urban sprawl. Uncontrolled urbanization endangers the palm groves, and results into significant environmental challenges (Dechaicha, 2020).
While significant progress has been made in understanding the benefits of urban green infrastructure, there are still several knowledge gaps that need to be addressed to optimize its implementation and management. Some of these gaps include its long-term effectiveness, scale and context, relation to equity and social justice, management and maintenance issues, metrics and evaluation. While urban green infrastructure is generally viewed as a positive and necessary part of urban planning and design, there are some controversies and challenges associated with its implementation. Some of these include its costs, maintenance, equity issues, effectiveness, and trade-offs.
In this context, the present study explores the dynamics of oasis green infrastructure in Biskra, Algeria, from pre-colonial times to the contemporary period. These infrastructures have their origins in palm groves, which fulfill economic, environmental, and social functions. The analysis aims at highlighting the crucial role of palm groves in urban sustainability, addressing also some of the gaps identified in the literature review.

MATERIALS AND METHODS

The study employs an approach involving the use of different types of data including detailed historical records, precise geographic information and environmental assessments. This method allows for an examination of the development of infrastructures throughout important historical periods like pre-colonial times the colonial era and the post-independence period.
By focusing on measures such as the size and variety of infrastructures, researchers can accurately measure the changes that have taken place over time.
Moreover, a classification of infrastructures was carried out to understand the environmental advancements during each historical era. This classification differentiates between the purposes and uses of infrastructures across time, offering a detailed insight into their progression and significance within specific historical and geographical contexts.
This analysis is carried out from a perspective on how individuals engage with their environment, emphasizing the importance of reflecting on the past to guide future decisions concerning sustainable development and natural resource management.

RESULTS AND DISCUSSIONS

Before the arrival of the Turks in 1541, Biskra was primarily characterized by an agricultural oasis, where 90% of the land was occupied by a vast palm grove. However, the arrival of the Turks prompted an acceleration of urbanization, leading to the creation of seven villages within the palm grove. This phase of development, marking the beginnings of ancient Biskra, resulted in a reduction of the palm grove area to approximately 80% of its initial size (Benmechiche et al., 2021). The seven oasis villages are ingeniously arranged around the mosque and Seguias (irrigation canals) that wind along the streets to water the palm grove gardens. The palm grove and Seguias played a central role in the formation of small human settlements in Biskra, while water was the driving force that shaped the structure of the city and its urban configuration in Biskra (Alkama, 2005).

During this pre-colonial period (before 1541-1844), the oasis green infrastructure in Biskra originates from the lush vegetation of the palm grove. It assumes a crucial role as habitat cover, thereby forming a bioclimatic envelope. This zone extends beyond providing thermal comfort. It also holds significant economic importance, as the palm grove serves as the foundation and life source of the region due to the versatility of ecosystems (Addad and Zerouala, 2002; Badache, 2014).

The thriving oasis ecosystem of Biskra owes its success to the interconnectedness of water, palm trees, and the human environment. This symbiotic relationship yields myriad benefits for the community of Biskra (Bouzaher and Alkama, 2017).

At the urban level, Biskra was characterized by a unique urban configuration, with houses integrated at the heart of the palm grove. Each village has its own characteristics, such as an exclusive watercourse, a central mosque, and a lively public square. Gardens also adorn some residences, adding a touch of beauty to the environment. This spatial organization shaped the morphological aspect of the city, in harmony with the climatic conditions (Hadagha, 2022). The streets and alleys of the Biskra offer a variety of landscapes and unique features. However, they all share a calm and serene atmosphere, enhanced by the presence of vegetation providing constant and soothing shade along these pathways (Figure 1). This atmosphere, in turn, fosters social interactions among the residents of the area (Farhi and Hadahga, 2018).

Agriculture and date palm cultivation play a fundamental role in local development. These activities help shape the environment and significantly improve the quality of life for residents.

Figure 1. The streets and alleys of the old city of Biskra (Source: Authors, 2024)

Additionally, they foster a harmonious connection between agriculture, date palm cultivation, and commerce, generating a social and economic fabric that contributes to the well-being of the local population. Biskra has leveraged its strategic geographical location by specializing in trade, adding an additional layer of prosperity to local life. Date palms, cereal crops, horticulture, and medicinal plants have played a key role in local development, creating a positive economic and social dynamic (Bouzaher and Alkama, 2017).

The traditional city of Biskra had a rich biodiversity due to the overlapping of three layers of vegetation creating an oasis effect. The palm grove provides for an ecosystem with a high biodiversity, serving as a refuge for numerous plant and animal species. This biological diversity plays a vital role in the ecological balance of the oasis, while also ensuring the livelihoods of the residents (Hadagha et al., 2018).

The inhabitants of Biskra, known as the Bsakra, had a variety of agricultural resources that allowed them to meet their economic needs (Figure 2). They had a palm grove, an olive grove, fields of wheat or barley, vegetable crops, a poultry yard, a sheepfold, and even a stable for donkeys (Zerdoum, 2003).

Figure 2. Variety of agriculture in an oasis of Biskra (Source: Authors, 2024)

Social cohesion is present where the palm grove acts as a central gathering place for all oasis residents. It serves as a hub for planning, recruiting, exchanging agricultural goods, and discussing the future of the oasis and their children. This area is where social bonds are forged, reinforced, and sustained over time, fostering a community life grounded in fairness and marked by diverse forms of solidarity (Farhi and Hadahga, 2018). A concrete example of solidarity within the palm grove is illustrated by the tradition of building oasis houses, called "Twisa". When one of the families in the oasis needs a new house or renovation, all residents come together to offer their support. This solidarity is demonstrated by collective mobilization to gather the necessary construction materials and actively participate in all stages of the construction process. Each member of the community contributes, whether by providing materials, offering labor, or assisting in coordinating efforts. This practice demonstrates a beautiful display of cooperation and mutual assistance within the community life of the oasis, strengthening social ties and enhancing the sense of belonging to the community (Farhi and Hadahga, 2018).

Ecologically, every part of the palm tree is utilized in a sustainable manner, thereby contributing to the balance of local ecosystems. Its wood is carefully employed in house construction, a traditional practice that has passed the test of time in sustainability terms (Figure 3). Additionally, the leaves of the palm tree hold considerable ecological importance. They are utilized in crafting fences, offering an eco-friendly alternative to synthetic materials or products sourced from deforestation (Bouzaher and Alkama, 2012; 2013).

Figure 3. Different applications of palm trees include their use: lintels, beams, fences and false ceilings (Source: Authors, 2018)

The palm grove plays a truly remarkable role in regulating the local climate. Acting as a natural shield against the sun, it provides protection against the region's high temperatures. This vegetative canopy also serves as an effective barrier against the hot winds from the south and the cold winds from the north, thereby creating a stable and comfortable atmosphere within the inhabited area. The presence of Seguia is also essential; it supplies water for crop irrigation, and also plays a crucial role in humidifying the dry air and regulating ambient humidity. Overall, these combined factors contribute to making the urban environment cooler and more pleasant, thereby enhancing the quality of life for the region's residents. The palm grove, with its irrigation system and dense vegetation cover, thus constitutes a fundamental element of Biskra's natural architecture, offering not only ecological benefits but also tangible advantages for the health and well-being of its inhabitants (Bencheikh, 2001).

The oasis ecosystem in Biskra demonstrates a remarkable ability to recycle and reuse waste highly efficient. This practice not only contributes to environmental preservation, but also improves soil quality and boosts agricultural productivity. The residents of Biskra have truly developed an ingenious approach to maximizing the use of available resources. It serves as an inspiring example of sustainability and nature conservation, underscoring the importance of harmony between humans and their environment (Hadagha, 2022).

During the French colonial period between 1844 and 1962, the city of Biskra underwent an interesting evolution in its development, including the change of vernacular architecture into a modern one, along with urban plans that cut off right streets and planted them alongside, creating other green space, in the nucleus of the new colonial city near the old one (Abdou and Alkama, 2022). Although the spirit of the oasis remains present with water, vegetation, and traditional architecture, it has evolved in a unique way. The oasis green infrastructure that once surrounded the city is now integrated into plots (Badache, 2014). By then, urbanization intensified, reducing the area of palm grove to 70% (Benmechiche et al., 2021). New types of green infrastructure, such as public gardens and the Dufourg square, were introduced. Biskra became a picturesque and tourist destination for

European vacationers. These gardens, such as the Landon garden and the public garden, were laid out, crossed by Seguias, and benefited from a traditional irrigation system that recalls the atmosphere of a real oasis (Naceur, 2004).

These gardens played a crucial role in regulating the temperature of the city of Biskra. Their balanced arrangement throughout the city favored the creation of a favorable microclimate, providing appreciable thermal comfort, which is particularly important in a hot and arid region like Biskra (Badache, 2014). Moreover, they were meeting places for various people and played an essential role in social life (Benmechiche et al., 2021).

Since the independence of Algeria in 1962, the city has experienced exponential urban growth (Figure 4), with a remarkable rate estimated at 63.6% (Kouzmine and Fontaine, 2018).

Figure 4. Current state of the oasis green infrastructure in Biskra (Source: Google Earth, 2024)

This uncontrolled urbanization has put the palm grove at risk leading to a decline of 66.56%, between 1985 and 2015, which poses significant environmental challenges. This underscores the significant impact of urban expansion on the oasis potential of the palm grove (Dechaicha, 2020). This phenomenon has caused a local climate change and an imbalance in the ecosystem of the city of Biskra; for example, in 1959, the temperature in the city center was 36°C, while around the palm grove it was 32°C. By 1999, these temperatures increased to 44°C and 32°C respectively (Adad and Zerouala, 2002). Furthermore, it has led to the emergence of the urban heat island in some areas place effect due to the presence of dense urban areas (Hamel et al., 2021).

The transition from an essentially agricultural society to an increasingly industrial one has disrupted the long-established economic balance in Biskra. Some owners are now opting to abandon palm trees, considering date farming not profitable enough (Adad and Zerouala, 2002; Hadagha, 2022).

The deterioration of the palm grove's irrigation systems, particularly the Seguias, has affected the regulatory structure of the urban fabric and the economic activity of the palm grove. These systems are essential for preserving both the ecosystem and the local economy (Berghout, 2015).

The pollution of the Seguias are a concerning issue in Biskra (Figure 5). Wastewater and household waste dumped into them can cause significant pollution. Additionally, the mixing of plant waste with urban waste complicates waste management. Over the years, the amount of urban waste in Biskra has increased, reaching around 100,044.13 tons in 2013, with approximately 80% being organic waste (Hadagha, 2022).

Figure 5. Deterioration and pollution of the Seguias (Source: Authors, 2024)

The results show that during the historical ages, due to different reasons, the urban green infrastructure of Biskra suffered from the anthropogenic pressure, reducing its total area and connectivity. In arid environments, green areas have a great importance as reservoirs of biodiversity, as indicated by other studies carried out in Algeria (Aouissi et al., 2022). At the same time, the processes contributing to the dynamics of oasis green infrastructure are also present in the European cities. Petrişor et al. (2021; 2022) found out the same in all Polish and Romanian cities during 1990-2018. A comparative study revealed that derogatory urban planning is common to Romania and Algeria (Hamma and Petrişor, 2018), and accounts for the urban sprawl and other deviations from the provisions of urban plans, especially in conjunction with social issues (Siboukeur et al., 2023).

Apart from making a series of recommendations, in line with previous research (Petrişor et al., 2021, 2022), for planners to preserve and expand the palm grove while maintaining its spatial continuity, for city managers and policy developers to rely more on using the results of research in decision-making and develop specific policies and for scientists to draft planning manuals, there are specific approaches involving initiatives such as rehabilitating irrigation systems, promoting organic agriculture, and raising awareness about environmental protection among residents. By fostering a harmonious coexistence between urbanization and ecological conservation, these strategies can contribute to preserving the palm grove of Biskra for future generations while supporting its sustainable development.

This study brings additional evidence that the green infrastructure needs to be accounted for in the planning process in order to maintain its spatial continuity and ability to provide ecosystem services (Legutko-Kobus et al., 2023), and its value consists of bringing such evidence from an area less explored in the literature (Aouissi et al., 2022). However, the implications expand far beyond the case study area, to other arid areas. Due to this natural feature, the study is relevant under the current climate changes debates, as green areas may help mitigating the effects of climate changes, at least locally (Gopinath et al., 2023; Singh and Kikon, 2024), and brings additional evidence for the connection between land degradation and climate changes (Corcheş, 2023).

The possible methodological limitations are due to the lack of data, especially of geospatial data (Udvardy et al., 2023), confining the analyses to a qualitative approach. Future may be able to overcome this lack by using satellite imagery (Noby et al., 2023). However, satellite imagery is able to reveal only the recent dynamics. There may be possibilities to combine data from different sources, although previous studies indicated that this approach yields inconsistent results, especially at a local level (Stângă and Niacşu, 2016; Stângă et al., 2016).

CONCLUSIONS

The evolution of oasis green infrastructure in Biskra reflects the contemporary challenges faced by many cities worldwide. With increasing urbanization and rapid development, natural ecosystems such as the palm grove are often threatened. However, these ecosystems play a crucial role in regulating local climate, conserving biodiversity, and providing essential ecosystem services.

In this context, the palm grove of Biskra represents a valuable ecological and cultural heritage. Its preservation is of paramount importance to ensure urban sustainability and maintain local identity. The challenges it faces, such as degradation of irrigation systems and pollution, require effective preservation strategies.

The historical analysis of Biskra reveals that urban sprawl, which can be connected to derogatory planning, is responsible for the loss of palm grove. From this perspective, several solutions can be foreseen, not excluding each other. One is developing planning regulations that account for the green infrastructure of Biskra and its spatial continuity, including the creation of new urban greenery, and the protection and connection of existing parts. These plans should also be enforced in a strict way. The second solution is educating people, including key actors (such as planners and administrators) and involving the dwellers of the city in the planning process.

Integrating sustainable practices into palm grove management is essential to ensure its resilience in the face of growing urban pressures.

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IN VITRO PROPAGATION OF *SAINTPAULIA IONANTHA* WENDL. GENOTYPES AND ASSESSMENT OF GENETIC STABILITY OF REGENERATED PLANTS USING CDDP MARKERS

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Abstract

A micropropagation protocol via direct shoot organogenesis from leaf explants of six commercial varieties of Saintpaulia ionantha Wendl was established in this study. The shoot induction was successfully achieved on Murashige and Skoog (MS) media supplemented with 0.2 mg L^{-1} indole-3-acetic acid (IAA) and 0.5 mg L^{-1} benzylaminopurine (BA). In proliferation stage, the effects of two combinations of PGRs (V1-0.2 mg L^{-1} IAA + 0.2 mg L^{-1} BA and V2-0.2 mg L^{-1} NAA +1 mg L^{-1} BA) on shoot number and length were examined for each genotype. The results suggest that PGR's combinations significantly influenced shoot proliferation in all analysed variety and among the treatments 0.2 mg L^{-1} NAA in combination with 1 mg L^{-1} BA was the most effective for in vitro shoot multiplication. The in vitro rooting percentage was 86.86-96.66% and was varieties-dependent. In vitro-raised plants showed a very high rate of survival (82-94 %). The genetic fidelity between the selected vitro-plants and mother plants were confirmed by CDDP markers.

Key words: *in vitro culture, African violets, molecular markers, genetic fidelity.*

INTRODUCTION

Saintpaulia ionantha H. Wendl., commonly known as the African violet (Moore, 1957), is a species that includes varieties showing a wide range of morphological characteristics with ornamental value. This species from the Gesneriaceae family is cultivated worldwide as a houseplant by flower enthusiasts, becoming an important asset to the floriculture industry. Visual appeal, compact size, attractive flowers and leaves, shade tolerance, and the ability to bloom under artificial light are some characteristics that make Saintpaulias popular potted houseplants (Ghimire and Fang, 2023; Grout, 1990). In the last century, the African violets were mainly propagated by seeds and leaf cuttings from the mature plants. These propagation techniques were considered time-consuming and usually, a small number of plants were regenerated from the mother plants (Preece, 2003). In this context, the development of valuable techniques for rapid propagation of African violets in a short time and limited space has been an important goal for scientists and floriculturists (Torres, 1989; Maghami, 2003). The capacity of African violets for easy regeneration has been extensively explored in vitro (da Silva et al., 2016). Thus, organogenesis via in vitro regeneration have been reported from different types of explants such as lamina leaves or leaves with petioles, petioles, internodes, floral buds, anthers, and sub-epidermal tissue (da Silva et al., 2017). It is worth noting that micropropagation has become a reliable approach for the commercial propagation of African violets, with an important objective of producing a large number of new and true-to-type plants in a relatively short time (Sharma and Kathayat, 2021; Sunpui and Kanchanapoom, 2002). However, the environmental conditions of in vitro culture and its duration may induce somaclonal variations in micropropagated plants which can lead to a reduced commercial value (Missaghi et al., 2023; Shukla et al., 2013). During *in vitro* culture, some genetic changes that occur at the molecular level can be expressed at the phenotypic level, but sometimes cannot be identified and evaluated even in plants acclimatized under *ex vitro* conditions (Hârţa et al., 2018). Nevertheless, DNA-based molecular markers can be successfully used for evaluating the genetic stability of in vitro cultivated plants of Gesneriaceae (Hârţa and Clapa, 2022). Among DNA-based molecular marker systems, Conserved DNA-derived polymorphism (CDDP) marker system is based on PCR amplification using a single primer that acts as reverse and forward, relies mainly on the conserved regions and provides comprehensive genomic information (Char et al., 2023). Genetic homogeneity testing of *in vitro*-grown plants adds value to any technology used for commercial purposes (Saidi et al. 2018). Based on the above-mentioned context, the main objectives of the present research were: to determine the influence of plant growth regulators (PGRs) on the *in vitro* culture of six varieties of African violets, to acclimatize micropropagated plantlets and to evaluate themunder greenhouse conditions, and also to evaluate the genetic fidelity of *in vitro*-raised plants with their mother plants after the 4th successive subculture using CDDP molecular markers system.

MATERIALS AND METHODS

Plant material

In this study, six hybrid varieties of *Saintpaulia ionantha* were used as mother plants. Plant material was provided by a certified nursery in Mijdrecht (Holland) and was purchased from a flower store. Before starting the experiments of the present study, the mother plants were cultivated under greenhouse conditions (22°C; 80% humidity). The varieties of Saintpaulias used as a source of explants for the establishment of *in vitro* culture are shown in Figure 1.

Figure 1. The African violet varieties used from the ‘Voilà’ plant assortment(original images)

Establishment of in vitro culture

In vitro culture initiation was done following the methodology described by Hârţa and Clapa (2022). After 60 days of *in vitro* culture on the initiation medium, adventitious shoots proliferated from the leaf explants were divided and further multiplied at 21-day intervals with two passages on MS medium supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA to provide plant stock for subsequent stages of *in vitro* multiplication of each African violet variety used.

Shoot proliferation and organogenesis

At the proliferation stage, adventitious shoots were divided and transferred to 370 mL (v/v) culture jars containing MS medium solidified with Plant agar (0.6% w/v) and supplemented with two variants of PGRs: V1 - 0.2 mg L^{-1} IAA + 0.2 mg L^{-1} BA and V2 - 0.2 mg L^{-1} NAA + 1mg L^{-1} BA. Adventitious shoots were subsequently transferred in aseptic condition to culture jars and cultured (21 days/subculture) at 24 ± 1°C under fluorescent white light (33.6 μmol m^{-2} s^{-1}) conditions with a photoperiod of 16/8 h light and dark cycles. All the chemicals and reagents were purchased from Duchefa, BiochemieBV, Holland. The average number of shoots/explant and the average shoot length were recorded after four repeated subculture in V1 and V2 proliferation culture media.

In vitro rooting and acclimatization

After the 4th successive subculture, the proliferated shoots were separated and then were rooted using MS medium without hormones (V3) and½ MS medium supplemented with 1 mg L^{-1} IAA (V4). Twenty explants (obtained from the shoot induction and multiplication experiment) were inoculated to each formulation for each variety. Data were recorded weekly for four weeks and values presented as means ± standard deviation. The rooted plantlets were thereafter subjected to acclimatization process. Briefly, the plantlets were taken out from the culture jars, rinsed carefully with sterile distilled water, and then grown in transparent plastic containers filled with moistened perlite. After 42 days, the plantlets were transplanted individually into plastic pots (6 cm Ø) filled with a potting

mixture of peat, vermiculite and perlite (2:1:1) and hardened under greenhouse conditions.
The average value of the survival rate for each analysed African violet variety was recorded after two months of growth in the greenhouse.

Genetic stability assessment
To confirm that the micropropagated plantlets were genetically true-to-type to their mother plants, genetic fidelity assessment by CDDP molecular markers was performed. Genomic DNA was isolated from fresh leaves weighing 10 mg. The ex-vitro mother plants and a total of five randomly chosen *in vitro* regenerated and subsequently greenhouse-grown plantlets from each variety analysed were used.
The extraction of total genomic DNA was performed using a Quick-DNA Plant/Seed Miniprep kit (ZymoResearch, USA) following the protocol described by the supplier company. Prior to CDDP analysis, DNA samples were diluted to 50 ng μL^{-1} using sterile double distilled water. Six CDDP primers were used to amplify DNA from each analysed African violets varieties and to confirm the genetic uniformity *of vitro*-plants with their mother plants. The six primers used generated detectable fragments in all samples analysed. To ensure the reproducibility of results, all PCR reactions were repeated twice. PCR was performed using a thermocycler system (SuperCycler Trinity by Kyratec, Australia) in 15 µL of PCR mixture containing 3 µL gDNA, 5.6 µL nuclease free H_2O for the PCR reactions, 2.5 µL GoTaq Flexi Green buffer, 2.5 µL MgCl2, 0.25 µL dNTP mix (Promega, USA), 1 µL CDDP primer (GeneriBiotech, Czechia), and 0.15 µL of GoTaq polymerase (Promega, USA). The PCR amplification process was started at 94°C for 5 min, followed by 35 cycles of 94°C for 60 s of denaturation, 60 s of annealing at 50°C, 120 s of extension at 72°C, and final extension at 72°C for 7 min. Separation of PCR products was performed by electrophoresis on 1.6% agarose gels (Promega, USA) stained with RedSafeTM Nucleic Acid staining solution (iNtRON Biotech, South Korea) in 1X TAE (Tris-acetate-EDTA buffer), at 80V and 88 mA for 2.5-3 hours. The electrophoretic bands profiles were visualized in UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany).

Data analysis
The experiment was arranged in a completely randomized design (5 replication x 5 inoculums/each variant). One-way ANOVA was performed to check the differences between the experimental variants. When the null hypothesis was rejected, post-hoc Duncan test ($\alpha < 0.05$) was used to separate and highlight the differences among means. The values shown are means ± S.E. CDDPs gel images were analysed using TotalLab TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to count the number and the range size of the amplified bands.

RESULTS AND DISCUSSIONS

Shoot induction and organogenesis
In this study, the induction of adventitious shoots (rosettes) from leaf explants of six Saintpaulia varieties, grown under *in vitro* conditions, was successfully done using MS medium supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA (data not shown). These results are consistent with those reported in other studies that revealed the high capacity of African violet leaves for in vitro shoot induction (Cassells and Plunkett, 1984; Shukla et al., 2013). In the present study, two different combinations of PGRs were tested to analyse the influence of phytohormones on the shoot proliferation stage. Thus, the PGRs combinations significantly influenced shoot proliferation in all analysed African violet varieties (Table 1).

Table 1. The influence of PGRs on the multiplication rate of proliferated shoots (number of shoots/inoculum) of the analysed varieties of African violets

Varieties	Multiplication rate V1 MS+0.2 mg L^{-1} IAA+0.2 mg L^{-1} BA	Multiplication rate V2 MS + 0.2 mg L^{-1} NAA+1 mg L^{-1} BA
Voilà Light pink	9.80 ± 0.26 D[8]	16.87 ± 0.24 F
Voilà Dark pink	8.80 ± 0.22 BC	15.45 ± 0.24 E
Voilà Dark blue	8.66 ± 0.23 B	17.09 ± 0.11 FG
Voilà Light blue	9.49 ± 0.20 CD	17.64 ± 0.21 G
Voilà Red	6.97 ± 0.13 A	16.62 ± 0.20 F
Voilà Pink-white	8.35 ± 0.18 B	16.51 ± 0.25 F

*The values shown are means ±SE. Different letters indicate significant differences between the variants according to Duncan's test.

The results show that the addition of cytokinins such as benzylaminopurine (BA) at a concentration of 1 mg L^{-1} and 0.2 mg L^{-1} auxins

(NAA) in MS medium had a stimulatory effect on the multiplication of Saintpaulia's adventitious shoots. As shown in Table 1, the aforementioned PGRs combination (V2) was found to be the best variant for shoot proliferation and significantly improved the number of shoots/inoculum for the blue-flowered cultivars named 'Voilà Light blue' (17.64 ± 0.21) and 'Voilà Dark blue' (17.09 ± 0.11). On the same variant of the culture medium (V2), non-significant differences were recorded between 'Voilà Light pink' (16.87 ± 0.24), 'Voilà red' (16.62 ± 0.20) and 'Voilà Pink-white' (16.87 ± 0.24) varieties. The lowest number of shoots/inoculum was recorded in the variety with dark–pink flowers (15.45 ± 0.24). High rates of direct shoot organogenesis were observed on V2 for all African violets varieties, indicating the genotype dependence of the PGRs combination (Figure 2).

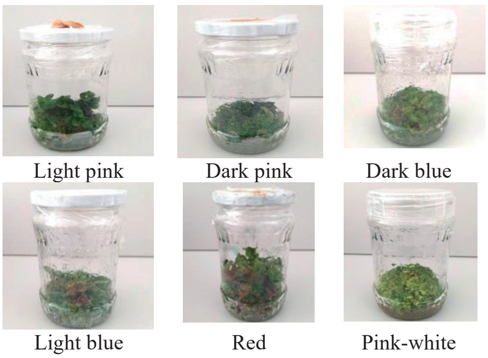


Figure 2. *In vitro* shoot proliferation of six African violet varieties on MS medium supplemented with 0.2 mg/L^{-1} NAA and 1mg/L^{-1} BA on 4th successive subculture

Thus, the results of the present study are in agreement with those reported by other authors (Sunpui et al., 2002; Shukla et al., 2013) who stated that MS medium containing BA in concentrations between 0.2 to 2.0 mg L^{-1} and NAA (0.1-0.2 mg L^{-1}) led to the highest efficiency of shoot proliferation per inoculum. High rates of direct shoot organogenesis were observed for all varieties, indicating that the genotype was dependent on the PGRs combination (Figure 2). Another important parameter of *in vitro* grown plants is the length of shoots. As shown in Table 2 significant differences were observed between the two variants of PGRs used (V1 and V2) in the case of all the analyzed varieties.

Table 2. The influence of PGRs on the length of proliferated shoots for analysed varieties of African violets

Varieties	Shoot length (cm)	
	V1 MS+0.2 mg L^{-1} IAA+0.2 mg L^{-1} BA	V2 MS + 0.2 mg L^{-1} NAA+1 mg L^{-1} BA
Voilà Light pink	1.48 ± 0.1 A[8]	1.97 ± 0.03 B
Voilà Dark pink	1.47 ± 0.12 A	2.01 ± 0.08 B
Voilà Dark blue	1.94 ± 0.08 B	2.18 ± 0.02 BC
Voilà Light blue	1.97 ± 0.05B	2.41 ± 0.04 CD
Voilà Red	1.67 ± 0.04 A	2.15 ± 0.04 B
Voilà Pink-white	2.00 ± 0.07 B	2.53 ± 0.09 D

*The values shown are means ±SE. Different letters indicate significant differences between the variants according to Duncan's test.

In this study, MS + 0.2 mg L^{-1} NAA+1mg L^{-1} BA was the best option for the elongation of Voilà Pink-white cv. shoots (2.53 ± 0.09 cm). The lowest shoot length value was recorded on the Voilà Light pink (1.97 ± 0.03 cm). This finding is consistent with the results reported by us in a previous study regarding the influence of PGRs combination on in vitro shoot growth from Gesneriaceae species (Hârţa and Clapa, 2022). Moreover, Daud and Taha (2008) reported that the proliferation and shoot growth of African violets from floral explants were also stimulated when MS medium was supplemented with 1 mg L^{-1} BA and 2 mg L^{-1} NAA. These results indicated the importance of auxin (NAA) as a plant growth regulator that stimulates both the number and length of African violet shoots grown *in vitro* conditions.

Rooting and acclimatization of plants

In vitro rooting of multiple shoots (rosettes) was recorded on MS medium without hormones (V3) and ½ MS+1mg L^{-1} IAA (V4) for all of six varieties of African violets used in the present study. The results summarized in Table 3 show that ½ MS supplemented with IAA stimulated the rooting of regenerated shoots for all six cultivars of *Saintpaulia ionantha*. Moreover, there were significant differences between cultivars regarding in vitro rooted shoots on the V4 variant except for the two genotypes named: Voilá Light pink and Voilá Dark blue. Data were recorded weekly for four weeks and values were presented as means ± standard deviation (Table 3).

Table 3. The influence of PGRs on the *in vitro* rooting of shoots for analysed varieties of African violets

Varieties	*In vitro* rooting (%)	
	V3 MS without hormones	V4 ½ MS + 1 mg L^{-1} IAA
Voilà Light pink	85.64 ± 0.16 A[8]	87.01 ± 0.01 AB
Voilà Dark pink	88.55 ± 0.11 A	89.48 ± 0.09 C
Voilà Dark blue	86.03 ± 0.29 A	86.94 ± 0.16 A
Voilà Light blue	88.58 ± 0.12 BC	89.44 ± 0.11 C
Voilà Red	93.55 ± 0.11 D	94.44 ± 0.12 D
Voilà Pink-white	94.66 ± 0.16 D	97.66 ± 0.29 E

*The values shown are means ±SE. Different letters indicate significant differences between the variants according to Duncan's test.

It is noted that, for some varieties analysed (Voilà Dark blue and Voilà Red) there were no significant differences between the percentage of root induction on V3 and V4 rooting media (Table 3). Similar response was observed in two previous studies of *Saintpaulia ionantha*. (Shajiee, 2007; Zeljković et al., 2021). These results might be due to the fact that the in vitro-grown plantlets of African violets produced a sufficient amount of auxins endogenously and no exogenous auxins were required for the induction of roots (Khan et al., 2007).

Regarding the acclimatization process, it is noteworthy that more than 82% of in vitro growth plants of the six analysed African violet varieties survived (Table 4).

Table 4. The survival rate of *in vitro* raised plants of the analysed varieties of African violets

Varieties	The survival rate (%)
Voilà Light pink	82.22 ± 0.01 A[8]
Voilà Dark pink	88.66 ± 0.11 B
Voilà Dark blue	86.88 ± 0.29 AB
Voilà Light blue	94.44 ± 0.12 C
Voilà Red	93.44 ± 0.11C
Voilà Pink-white	94.66 ± 0.16 C

*The values shown are means ±SE. Different letters indicate significant differences between the variants according to Duncan's test.

It was observed that although the plantlets were sufficiently rooted, it is nearly mandatory for the plantlets that they should be kept one month covered with the plastic lids, prior to the direct exposure of the plantlets under greenhouse conditions.

After 1 month, raised-plants were successfully transferred in greenhouse, followed by normal plant growth and flower development. In a previous study, also, Khan et al. (2007) observed that although African violet plantlets were sufficiently rooted, it was almost mandatory to be kept in a tunnel covered with a plastic sheet, before direct exposure of the plantlets under greenhouse conditions. The period required to keep the plants in the tunnel varied with the season and suggested that the best time for the transfer of plantlets for the acclimatization stage is the winter season.

Genetic stability assessment

Several strategies were followed to assess the genetic fidelity of the in vitro-raised progenies of ornamental plants since the sustainability of the micropropagation technique depends on ensuring the genetic fidelity of the regenerated plants (Alizadeh et al., 2015).

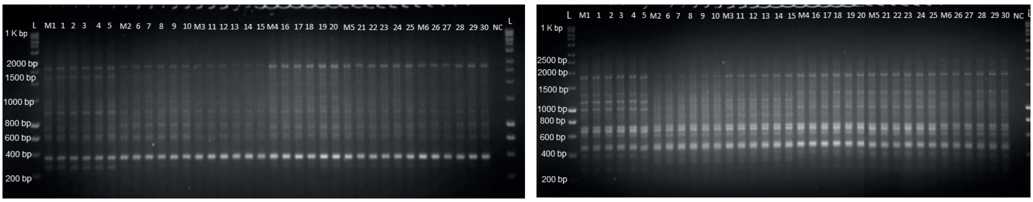
Despite the availability of abundant literature supporting the importance of large-scale multiplication of African violets using in vitro cultures, it is also very important to evaluate the genetic uniformity of acclimatized plants using molecular markers systems (Hârţa and Clapa 2022; Tyagi et al., 2022; Biswas and Kumar, 2023). To confirm the uniformity of in vitro grown plants with their mother plants, CDDP markers were employed to analyse the genetic fidelity of five randomly selected acclimatized plants from each variety. All CDDP primers used generated clear and detectable bands for all samples analysed (Table 5). The genetic analysis with CDDP markers showed a total number of monomorphic bands between 64 (Voilà Dark blue) and 70 (Voilà Light blue) as shown in Table 5. Each CDDP markers generated monomorphic bands and their length ranged between 200 and 2100 bp.

Moreover, no genetic differences were observed in any of the analysed African violets variety.

As shown in Figure 3 *in vitro* raised plants have the same banding patterns as the mother plants, indicating their uniformity at the DNA molecular level.

Table 5. Sequences of CDDP primers and the number of monomorphic bands generated in the analysed Gesneriaceae cultivars

Primer name	Primer sequence 5'-3'	Number of monomorphic bands						Range size of bands (bp)
		Voilà Light pink	Voilà Dark pink	Voilà Dark blue	Voilà Light blue	Voilà Red	Voilà Pink-white	
Knox 1	AAGGGSAAGCTSCCSAAG	9	8	7	10	9	10	300-2100 bp
WRKY-R3B	CCGCTCGTGTGSACG	12	11	11	12	13	9	380-2100 bp
Myb1	GGCAAGGGCTGCCGC	13	13	14	13	12	13	200-1800 bp
Myb2	GGCAAGGGCTGCCGG	12	12	11	12	12	13	200-2000 bp
ERF1	CACTACCCCGGSCTSCG	10	11	11	12	11	11	300-1600 bp
ERF2	GCSGAGATCCGSGACCC	9	10	10	11	12	9	300-1800 bp
Total		65	65	64	70	69	65	



(a) (b)

Figure 3. PCR banding patterns obtained with Knox1 (a) and WRKY-R3B (b) CDDP primers after the PCR amplification of African violets varieties: Voilà Light pink (M1; 1-5); Voilà Dark pink (M2; 5-10); Voilà Dark blue (M3; 11-15); Voilà Light blue (M4; 16-20); Voilà Red (M5; 21-25) and Voilà Pink-white (M6; 26-30). Lane L-1kb DNA Ladder; M-mother plants; NC-negative control

Although genetic fidelity has been assessed by various DNA-based molecular marker systems in African violets (Biswas and Kumar, 2023), to our knowledge, this is the first paper to report the use of the CDDP marker system to evaluate the genetic uniformity of African violet varieties.

CONCLUSIONS

This research demonstrated that direct plant regeneration of *Saintpaulia ionantha* Wendl. through leaf explants was successfully achieved, but is cultivar dependent. The genetic fidelity between the selected vitro-plants and mother plants were confirmed by CDDP markers.

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VARIABILITY STUDIES OF LOCAL POPULATIONS OF *ANACAMPTIS LAXIFLORA* SSP. *ELEGANCE* LAM. SSP. *ELEGANS* (HEUFFEL) SOÓ FROM THE OLTENIA REGION, ROMANIA

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Abstract

Orchids are some of the most popular flowers. They are appreciated for their beauty and the varied range of colors and shapes Anacamptis laxiflora ssp. elegans is a "gem of the Romanian landscape" that grows in meadows, forest edges and clearings. The unique characteristics of this orchid species are to be appreciated because of its morphological complexity. Comparative analysis of plant morphological traits indicates that there are significant differences between natural populations of this terrestrial orchid species. A special priority should be given to this type of orchid because they are very common in the habitats of the Oltenia region and have large populations. Their monitoring in the context of global warming is also necessary, and one of the conservation priorities concerns the protection of the habitats in which they occur. Regarding the analysis of character variability according to plant density, it influences the average plant length, stem diameter and leaf number/pl., so the values recorded for these characters have significant differences compared to the values recorded by plants at lower densities of individuals.

Key words*: Anacamptis laxiflora ssp. elegans, morphological characters, population, variability.*

INTRODUCTION

Orchids are among the most beautiful wonders of nature and are considered splendors of the plant world, growing in the warm areas of the planet, with very varied shapes and colors, as well as strongly scented.

Family *Orchidaceae* Juss. is the second largest family of the Plant Kingdom, after the *Asteraceae* Family (Bercht. & J. Presl), with about 750-850 spontaneous and cultivated genera, respectively 20,000-35,000 species and over 120,000 hybrids (Tomescu, 2018). The same author claims that orchids are found on all continents, except Antarctica, and the area of growth and distribution stretches between 54^0 south latitudes and 68^0 north latitudes, from the low plains, at altitudes of 2,000 and even 4,000 m, in territories from America, Asia, Africa, Australia, Oceania and Europe.

In our country there are orchids with smaller and more mysterious inflorescences and with very varied shapes. According to Ciocarlan (2009), the *Orchidaceae* family in Romania is represented by 58 species while Sârbu (2013), says that there are 25 genera of orchids, with 60 species, which are sometimes divided into subspecies according to the color of the corolla, the sizes of the various organs, etc. The best represented genera are: *Orchis, Dactylorhiza, Epipactis* with over 5 species each. Even within a genus, the shape of the flower can vary quite a bit, making it easy to differentiate the species. We observe this in the case of the genus *Orchis* (*Orchis militaris, Orchis purpurea, Anacamptis coriophora, Anacamptis laxiflora* ssp. *elegans, Orchis tridentata, Orchis ustulata*).

Wild orchids in Romania can be found on all landforms, from the highest rocky peaks with an altitude of 2,400 meters, to the sand dunes on the Black Sea coast. They grow on any type of substrate, covering the whole range of humidity, from the driest to the wettest, marshes, swamps or periodically flooded areas. Some orchids are sun-loving species, growing in full sun in short grass and hay meadows or meadows, while others are shade-loving and can grow hidden in the darkest woods.

Compared to other European countries where some species are very rare or have very low populations, in Romania most species are still well represented by numerous and relatively constant populations. They are quite special from a botanical point of view.

Due to the lack of education, in terms of environmental protection, many locations with native orchids have been destroyed, thus becoming one of the most endangered plant groups, both in our country and even worldwide. That why most of the orchid species from the Romania's flora are included in "The Red List of superior plants in Romania" as rare or in danger (Oltean et al., 1994). Orchids are mostly found in tropical areas, but Romania is also a favorable area for the development of orchids.
Long-term monitoring programs of orchid populations have shown that their dynamics are highly dependent on prevailing weather conditions, suggesting that changes in climatic conditions can have far-reaching effects on population dynamics and thus on orchid distribution (Evans et al., 2020).
Thus, there are limited studies related to the effects of site factors on the morphological and anatomical characters of the *Orchidaceae* family. The present study was carried out to bring new data on the variability of *Anacamptis laxiflora* ssp. *elegans* identified in the Băile Govora resort area. The areal is placed between Govora-Village and Bunești, Vâlcea county, Oltenia region, Romania. This is a sporadically encountered species and currently is charted only in a few sites in Oltenia, being subject to threats and anthropogenic pressures.
The species was identified in swampy meadows, in the meadow area of Govora River, between Bunești and Govora-Village. Following the studies, it was found that populations with numerous and very vigorous individuals were identified in the floristic composition of two plant communities: ***Poëtum trivialis*** Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* Soran 1962) (Resmeriţă et al., 1971) and ***Anthoxantho-Agrostetum capillaris*** (Sillinger 1933, Jurko 1969).

1. *Poëtum trivialis* Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* (Soran 1962; Resmeriţă et al., 1971).
Phytocoenoses of mountain sedge grow on lands with excess moisture in stream meadows, swampy or temporarily flooded places. It is a plant community that is less widespread in the Govora basin, being found in the hilly floor, within the radius of the Bunești locality. In the floristic composition of the phytocoenoses of this plant community, the analyzed species was found only in two.
In the floristic composition of the observed phytocoenoses, the following species enjoy a high constancy: *Festuca pratensis*, *Galium palustre*, *Oenanthe banatica*, *Ranunculus repens*, *Agrostis stolonifera*, *Filipendula vulgaris*, *Lythrum salicaria*, *Juncus inflexus*, *Symphytum officinale*, *Equisetum palustre*, *Poa pratensis*, *Anacamptis laxiflora* ssp. *elegans* etc.

2. *Anthoxantho-Agrostetum capillaris* Sillinger 1933, Jurko 1969.
Field grass meadows with sedge usually establish themselves on cleared, flat or gently sloping land in the hilly and montane floor, preferring typical eutricambosols or luvosols. In the Govora basin, such phytocoenoses were analyzed near the town of Govora-Village, at the border of the meadow forest habitats built by *Alnus gutinosa*. Phytocoenological observations for the knowledge of the vegetation in the Pesceana river basin, Vâlcea county, carried out by Măceșeanu & Făgăraș (2023) allowed the identification of 9 types of habitats of community interest, including the one referring to alluvial forests of *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*).
In the floristic composition of the phytocoenoses analyzed, along with the edifying species of the plant community, there are also numerous species belonging to the class *Molinio-Arrhenatheretea* such as: *Briza media*, *Trifolium repens*, *T. pratense*, *Luzula campestris*, *Vicia cracca*, *Poa pratensis*, *Holcus lanatus, Anacamptis laxiflora* ssp. *elegans* (Figure 1), etc.
Following the analyzes we carried out in the field, the species from the floristic composition of the phytocoenoses in the studied area are presented in Table 1.

Figure 1. *Anacamptis laxiflora* ssp. *elegans* in the Govora Stream basin (foto. M. Niculescu)

Table 1. The floristic structure of *Anthoxantho-Agrostetum capillaris* Sillinger 1933, Jurko 1969 plant community

No. of relevée	1	2	3	4	5	6	7	8	K
Altitude (x 10 m.s.m)	35	35	38	38	40	38	40	35	
Coverage of herbacaeous layer (%)	100	95	100	100	100	100	95	95	
Area (m^2)	100	100	100	100	100	100	100	100	
Character species of plant community									
Anthoxanthum odoratum	4	4	3-4	4	4	4-5	4	5	V
Agrostis capillaris	1	1	2	1	2	+	1	+	V
Molinietalia									
Agrostis stolonifera	+	+	-	-	-	-	+	+	III
Trifolium hybridum	-	-	+	-	+	+	-	-	II
Alopecurus pratensis	+	-	-	+	-	+	-	-	II
Dactylorhiza maculata	+	+	-	-	-	-	+	+	III
Myosotis scorpioides	-	-	+	-	+	+	-	-	II
Juncus articulatus	+	-	-	+	-	+	-	-	II
Juncus conglomeratus	+	+	-	-	-	-	+	+	III
Anacamptis laxiflora ssp. *elegans*	1	1	+-1	1-2	1	1-2	+-1	+-1	V
Molinio-Arrhenatheretea									
Briza media	+	+	+	+	+	+	+	+	V
Holcus lanatus	-	+	+	+	+	+	+	-	IV
Poa pratensis	-	+	-	+	-	-	+	+	III
Trifolium pratense	+	-	+	+	-	+	-	-	III
Trifolium repens	+	+	-	+	+	+	+	+	IV
Vicia cracca	-	-	+	-	+	-	-	-	II
Festuca pratensis	-	+	+	-	-	-	+	+	III
Centaurea austriaca	+	-	-	+	-	+	-	-	II
Ranunculus acris	-	+	+	-	+	+	+	+	IV
Dactylis glomerata	+	+	-	+	-	+	+	+	IV
Poa trivialis	-	+	+	-	-	-	+	+	III
Stellaria graminea	-	+	-	+	-	-	+	+	III
Luzula campestris	+	+	-	+	+	+	+	+	IV
Ononis arvensis	+	-	+	-	-	-	+	-	II
Polygala vulgaris	+	+	+	+	+	+	-	-	IV
Lychnis flos-cuculi	+	-	+	+	-	+	-	-	III
Achillea millefolium	+	-	+	-	-	-	+	-	II
Leontodon autumnalis	+	-	-	+	-	+	-	+	III
Leucanthemum vulgare	+	+	+-1	1	1	+	+	+	V
Lychnis flos-cuculi	+	-	+	+	-	+	-	-	III
Arrhenatheretalia									
Cynosurus cristatus	1	+	+	+	+	1	+	+	V
Rhinanthus rumelicus	+	-	+	+	+	+	+	-	IV
Carum carvi	+	-	-	+	-	+	-	-	II
Campanula patula ssp. patula	+	+	-	+	+	+	+	+	IV
Festuco-Brometea									
Carex caryophyllea	-	+	-	-	+	-	+	+	III
Galium verum	+	+	+	+	+	+	+	+	V
Danthonia provincialis	+	-	+	-	+	+	+	+	IV
Dianthus carthusianorum	+	+	+	+	+	+	+	+	V
Thymus pulegioides	-	+	-	-	+	-	+	+	III
Potentilla argentea	-	-	-	-	+	-	-	+	II
Prunella laciniata	-	-	-	-	+	-	-	+	II
Coronilla varia	+	+	+	+	+	+	+	+	V
Variae Syntaxa									
Vicia tetrasperma	-	-	-	-	+	-	-	+	II
Veronica chamaedrys	-	-	-	-	+	-	-	+	II
Vicia sativa	+	+	-	-	-	+	+	+	III
Rorippa sylvestris	+	+	-	-	-	-	+	+	III
Lychnis viscaria	-	+	-	-	-	-	+	+	II
Prunella vulgaris	+	+	-	-	-	-	-	+	II
Viola collina	+	+	-	-	-	+	+	+	III
Rumex crispus	+	+	-	-	+	-	+	+	III
Equisetum arvense	+	-	-	-	-	+	-	+	II
Cruciata laevipes	+	+	-	-	-	+	+	+	III

Place and data of the relevés: Govora Valley, Govora-Sat, 2.V.2022; 25.IV.2022
Source: performed by the authors based on the own research

MATERIALS AND METHODS

An investigation was carried out to study the variability of the morphological characteristics of *Anacamptis laxiflora* ssp. *elegans* population identified in Băile Govora area. The climate of the experimental site is mild, semi-Mediterranean. The air temperature shows small seasonal and diurnal variations, the lowest being in the cold season, when average monthly temperatures are +0.2^{0}C in December, -2.8^{0}C in January, +9^{0}C in February.

The heaviest winter days occur in the first half of January and 28 days of genuine winter are recorded annually. The record minimum temperature in the cold season was recorded on 11.02.1929, when it reached the value of -27.4^{0}C.

The warm season lasts more than 6 months, from April to October; the days with the maximum temperature above 25^{0}C start from April (9 days) and end in October (3 days) so that during the summer there are 88 days with a temperature of 25^{0}C. Tropical days with temperatures of 30^{0}C and rarely above, they meet in June, July and August, totaling 23 days. Characteristics are the absence of heat during the night, and autumns are warmer than springs. In this sense, the measurements showed values of the insolation coefficient that vary from 0.20 in the cold season to 0.54 in the warm season.

Atmospheric precipitation falls within normal annual limits, determining a pluviometric regime favorable to the development of vegetation. The most days with precipitation were recorded in the months of May - June (13 days) and in August - September, 5 days each. Massive downpours, followed by local floods, were recorded at long time intervals (15-25 years), these phenomenon being closely related to the global ones. The electrical state of the atmosphere has a potential of 42 V/m, the electrical gradient having values equal to or greater than 100 V, only when the weather is unstable and with electrical manifestations (https://www.primaria-govora.ro/clima).

In this study, the morphological characters were: plant height, stem diameter, leaf number, leaf width, leaf length, ratio of leaf width/length, inflorescence length, flower number, flower width, flower length and ratio of flower length/width. The analyzed plants were kept under careful observation during the determinations. Plants were verified using the classical determination method. For the identification were used Ciocârlan (2009) and Sârbu et al. (2013).

The obtained results were calculated using analyses of variance method. Statistical analyses were executed in Excel program. The significance of the difference was evaluated by the least significance difference (LSD 5%).

RESULTS AND DISCUSSIONS

The present study was conducted during the period from March to September 2022. The Băile Govora Resort area appears to be a suitable habitat where the distribution of orchid populations increases under conditions of mild climate change. Ilie et al. (2023) reported a decrease in the number of dryland loving species, largely represented by annual plants growing in open areas.

In Tables 2 and 5 the variability of the morphological characters of plants, respectively flowers, is presented for *Anacamptis laxiflora* ssp. *elegans*, depending on the plants density.

The obtained results show that the average height of the plant varied from 23.54 cm at 4 pl./m^2 to 73.99 cm at densities higher than 35 pl./m^2. The stem diameter varied from 3.72 mm at 25 pl./m^2 to 6.25 mm at densities higher than 35 pl./m^2. The average number of leaves presented smaller limits, between 6.67 at 4 pl./m^2 and 7.63 at densities higher than 35 pl./m^2. The length and width of the leaves varied from 15.8 mm at more than 35 pl./m^2 to 29.4 mm at 4 pl./m^2, respectively 2.6 mm at 25 pl./m^2 to 3.6 mm at 4 pl./m^2.

Intermediate values to those obtained in this research for plant height and stem diameter were reported by Anghelescu et al. (2020) for a population of 7 hybrids between two highly divergent species, *Anacamptis coriophora* (from *Anacamptis coriophora* group) and *Anacamptis palustris* subssp. *elegans* (from *Anacamptis palustris* group), species which are taking part from the same family as *Anacamptis laxiflora* ssp. *elegans*. Also, Niculescu et al. (2023) concluded that at *Anacamptis coriophora*, plant height is a character influenced by humidity, light and location.

For the character length and width of the leaf, values of 20 and 2.0 cm, respectively, were mentioned by Şeker (2022) for the taxon *Anacamptis laxiflora* (Lam.) R.M. Bateman, Pridgeon & M.W. Chase identified in a habitat at the edges of coniferous forests and meadows. Related to the analysis of character variability according to plant density, it influences the characters plant length, stem diameter and leaf number, so the values recorded for these characters record significant differences compared to the values recorded by plants at lower densities of individuals.

For the character leaf number and leaf width, the highest values are recorded for plants at low densities, these values recording significant differences compared to the values recorded for these characters at plants at high densities.

As variability coefficient point of view, the most variable character was leaf length followed by leaf width.

Table 2. Variability of *Orchis laxiflora* ssp. *elegans* morphological characters depending on plants density

Character / Statistical Index	Plant length (cm)		Stem diameter (mm)		Leaf number		Leaf length (mm)		Leaf width (mm)	
	Value	C%	Value	C%	Value	C%	Value	C%	Value	C%
4 pl./m^2	23.54±3.24^c	13.78	4.36±0.56^b	12.75	6.67±0.41^b	6.14	29.4±5.03^a	25.93	3.6±0.55^a	15.21
15 pl./m^2	34.30±1.14^b	3.31	4.09±0.78^b	18.97	6.96±0.49^b	7.04	27.0±7.00^a	24.89	3.2±0.84^a	21.07
25 pl./m^2	39.73±2.83^b	7.13	3.72±0.62^b	16.74	7.52±0.27^a	3.55	20.6±5.13^b	23.43	2.6±0.55^b	29.88
>35 pl./m^2	73.99±15.59^a	21.08	6.25±1.37^a	21.92	7.63±0.18^a	2.39	15.8±3.70^c	17.11	2.8±0.84^b	26.15
LSD 5%	7.11		1.08		0.351		4.22		0.56	

Related to the analysis of the trend model between the character average plant length and plant density, a coefficient of correlation of 0.862 was calculated, a value that demonstrates a very strong link between these two indices. In other words, the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.743, which implies that the trend model calculated based on the simple linear equation is a valid one. Thus, the value of the coefficient of regression calculated was equal to 1.513, which implies that when the plant density increases by individual/m^2, the average plant height/m^2 increases by 1.5 cm (Figure 2).

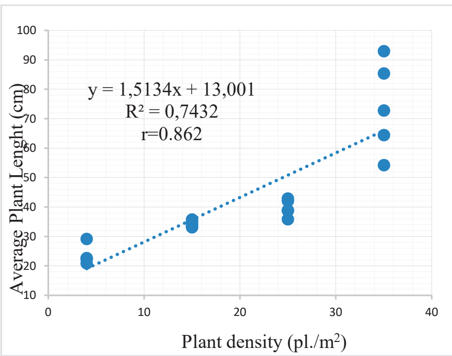

Figure 2. Correlation and trend pattern analysis between average plant length and plant density

As concern the analysis between the average stem diameter and plant density, the correlation coefficient was of 0.461 which indicates that there is no strong connection between these two indices (Figure 3). Between the character average leaf number/pl. and plant density, the coefficient of correlation of 0.755 demonstrates a strong link between these two indices. In other words, the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.5706, which means that the trend model calculated based on the simple linear equation is not a valid one (Figure 4). For the character leaf length and plant density, the coefficient of correlation of 0.732 demonstrates a strong link between these two indices, which indicate that the increase in plant density brings with it an increase in value for this character. The coefficient of determination had a value of 0.536, which implies that the trend model calculated based on the simple linear equation is not a valid one (Figure 5).

Related to the analysis of the trend model between the character average leaf width and plant density, a coefficient was calculated. of correlation of -0.456, a value that proves that there is no strong connection between these two indices (Figure 6).

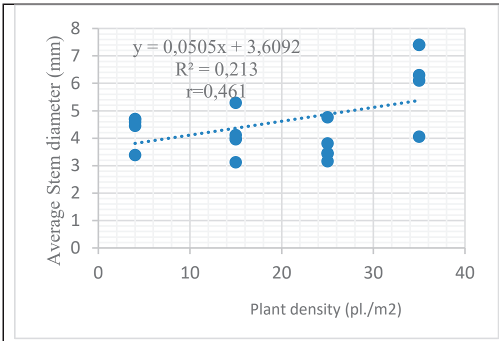

Figure 3. Correlation and trend pattern analysis between average stem diameter and plant density

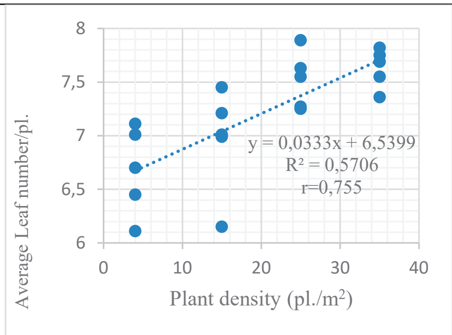

Figure 4. Correlation and trend pattern analysis between average leaf number/pl. and plant density

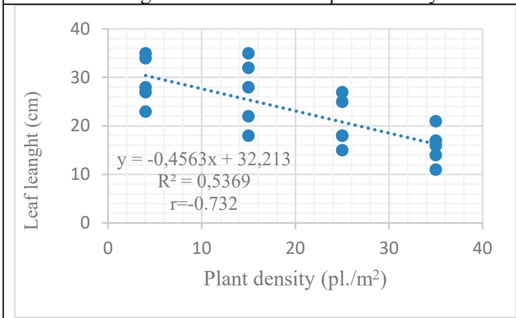

Figure 5. Correlation and trend pattern analysis between leaf length and plant density

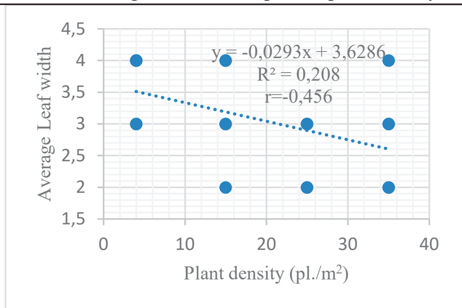

Figure 6. Correlation and trend pattern analysis between leaf length and plant density

Regarding the Principal Components Analysis, for the plant's morphological characteristics the first two components explain 93.918 % of total variance, the first component registering 56.007 % and the second component 37.911% (Table 3).

Table 3. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.800	56.007	56.007	2.800	56.007	56.007
2	1.896	37.911	93.918	1.896	37.911	93.918
3	0.304	6.082	100.000			
4	4.491E-016	8.983E-015	100.000			
5	-3.281E-017	-6.562E-016	100.000			
Extraction Method: Principal Component Analysis.						

Related to the first component, the studied characters that obtain high positive value are average leaf length, average stem diameter and average leaf length. The first component can also be named plant capacity to have well developed stem on which long leaves can be inserted. For the second component, high positive values are obtained by average leaf width. The second component can be named the ability of plants to develop leaves with large width (Table 4).

Table 4. Component Matrix-Extraction Method: Principal Component Analysis, 2 components extracted

Character \ Comp. value	Component	
	1	2
Average plant length	0.879	-0.476
Average stem diameter	0.989	0.019
Average leaf number	0.523	-0.800
Average leaf length	0.781	0.598
Average leaf width	0.409	0.819

The four groups based on the values of the two components were the following (Figure 7):

- the variant with the first component positive and the second one negative is the one with more than 35 pl./m^2 density. This variant has high values for the first component;
- the variants with both negative components are the ones with 15 and 25 pl./m^2 density;
- the variant with the first component negative and the second one positive is the one with 4 pl./m^2 density.

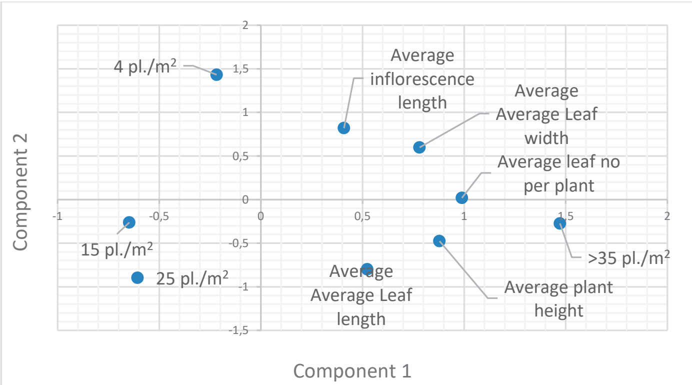

Figure 7. Classification of variants according to the score of the two components

Average length of the inflorescence varied from 98 mm at 25 pl./m^2 to 182 mm at 4 pl./m^2. The number of flowers varied from 15.6 at 4 pl./m^2 to 47.8 at over 35 pl./m^2. The length of the flowers varied more than their width (Table 5). Lower values for flower length and width than in this research were reported by Erzurumlu et al. (2018) in a phylogenetic relationship among fourteen different tuber-producing orchid species and after analyzing phenotypic and genetic variation within and among the natural population through fifteen morphometric traits and ten random amplified polymorphic DNA (RAPD) primer combinations.

The development of orchid populations is high in some sunny places and low in others. However, we appreciate this species as showing adaptive variation. These results indicate the direct and indirect effects of climate change on population viability. They are consistent with the data obtained by Sletvold et al. (2013). Therefore, it is recommended that the conservation studies of this orchid species be carried out on a limited geographical unit.

A. laxiflora is known as having a dense inflorescence and deeply lobed lip. The occasional hybrids between other members of the subgroup are often very robust, sometimes attaining 135 cm in height, with many-flowered inflorescences (Wood & Ramsay (2004). In another type of orchid, *Spiranthes spiralis,* average length of the inflorescences is strongly influenced by the height of the plant, meaning that tall plants usually have longer inflorescences (Niculescu et al., 2024).

Table 5. Variability of *Anacamptis laxiflora* ssp. *elegans* morphological flower traits depending on density

Character / Statistical Index	Inflorescence length (mm)		Flower number		Flower length (mm)		Flower width (mm)	
	Value	C%	Value	C%	Value	C%	Value	C%
4 pl./m^2	182±37.4[a]	20.55	15.6±2.22[d]	14.23	37.6±5.29[a]	14.07	4.4±0.62[a]	14.09
15 pl./m^2	135±22.5[b]	16.67	26.8±4.56[c]	17.01	29.2±4.55[b]	15.58	3.8±0.59[b]	15.53
25 pl./m^2	98±15.8[c]	16.12	33.17±5.11[b]	15.41	18.67±3.22[c]	17.25	2.63±0.33[c]	12.55
>35 pl./m^2	70±10.21[d]	14.59	47.8±8.25[a]	17.26	10.2±1.59[d]	15.59	2.52±0.29[c]	11.51
LSD 5%	11.78		7.81		6.81		0.43	

In this study, the dendrogram of the plants was made according to the density of the plants, based on the analyzed characters of the flower. Thus, the plants at the highest densities are the closest in terms of flower characters, which in turn are most similar to the plants at a density of 15 pl./m^2. The plants with the lowest density, in terms of flower characters, are the most different from the other plants (Figure 8).

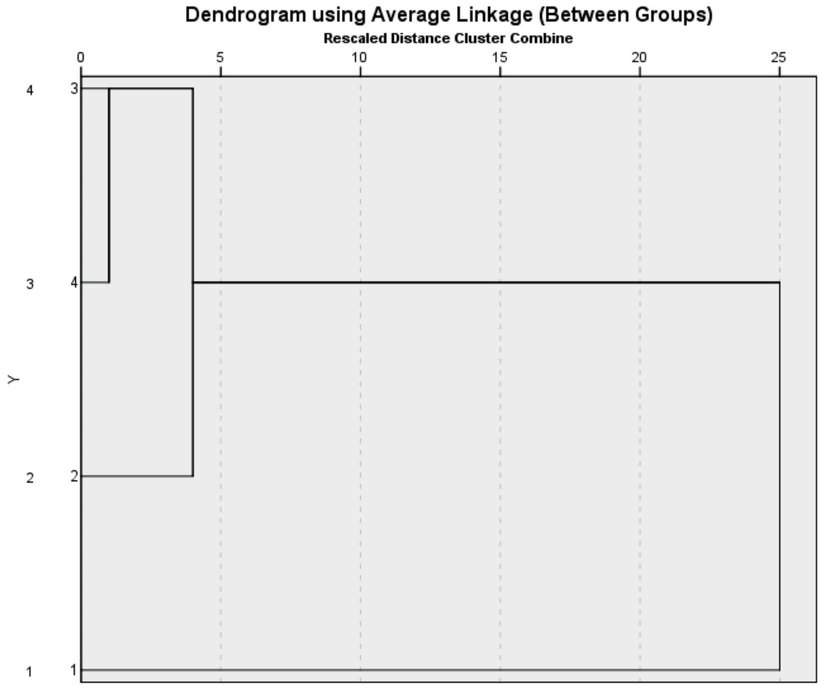

Figure 8. Dendrogram of the plants according to density

CONCLUSIONS

Anacamptis laxiflora ssp. *elegans* is a rare species and is recommended to be admired without being collected. We must let wild orchids grow in their natural habitats, otherwise we will only have artificially grown orchids.

From a cenotaxonomic point of view, this taxon was identified in the floristic composition of two plant communities: ***Poëtum trivialis*** Soó 1940 (Syn. *Agrosteto-Poëtum trivialis* Soó 1938, *Trifolio- Poëtum trivialis* (Soran 1962, Resmeriţă et al., 1971) and ***Anthoxantho-Agrostetum capillaris*** Sillinger 1933, Jurko 1969.

The abundance-dominance of the taxon within the analyzed phytocoenoses varies mainly according to the soil moisture level, being quite high, and the populations with increased density and vigor being found in the meadows of the Govora basin in the plant community ***Anthoxantho-Agrostetum capillaris*** Sillinger 1933, Jurko 1969.

The density of plants per square meter is a determining factor in terms of their evolution and development. Thus, plants at high density are the tallest compared to plants at lower densities, a situation that is also found in the analysis of other characters such as stem diameter or leaf no./pl. Also, low density positively influences average leaf length and leaf width.

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EFFECTS OF ORGANIC AND MINERAL FERTILIZERS ON GROWTH AND FLOWERING OF YOUNG LAVENDER (*LAVANDULA ANGUSTIFOLIA* MILL.) PLANTS

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Abstract

Although lavender grows in its native environment in rocky and poor soils, various studies have shown that it responds positively to fertilizers, increasing not only the yield but also the quantity and quality of the essential oil. In order to determine the most effective type of fertilizer - organic or mineral, young plants of the Sevtopolis lavender cultivar were tested in a field experiment conducted in the village of Răduleşti, Ialomiţa County, in south-east of Romania. Fertilizers were applied before vegetation, using either yard waste compost or a mineral fertilizer containing 11-11-21 macronutrients and micronutrients. The results showed that in the first year of growth, the mineral fertilized plants had grown less. Floral initiation and flowering period were not influenced by the type of fertilizers. The length of flowering stem and the size of inflorescence were smaller in case of mineral fertilization but the number of inflorescences was significantly lower in lavender plants fertilized with compost.

Key words: *chemical fertilization, Lavandula angustifolia Mill., phenology, sandy soil, yard waste compost.*

INTRODUCTION

Habitat conditions provide essential information about species preferences and establish a starting point for their cultivation in other regions. Although *Lavandula angustifolia* Mill. grows wild in Mediterranean region, characterized by hot and dry summers, followed by cool and wet winters, it showed a great adaptability to other climates (King et al., 2012; Jamoldinovich & Yorkulovich, 2021; Peçanha et al., 2021; Manushkina, 2023). However, before 2010, lavender was not a crop of interest for Romania (Vijulie et al., 2022), especially due to the winter frosts that partially or totally damaged the plants even in the warmest regions of the country. After this year, due to the climate changes and the introduction of new cultivars, it can be cultivated in almost any region of Romania. Lavender is more and more cultivated today, especially in the southern Romania (Vijulie et al., 2022), where dry and hot summers are followed by mild and poor in precipitations winters (Bogdan et al., 2008; Marinica & Marinica, 2019; Marinica et al., 2021; Nagavciuc et al., 2022). This dry climate restricted certain traditional crops, such as wheat or maize. But it is not only the extended periods of droughts and poor irrigation that forced farmers to take action and test other crops, but also the soil characteristics that are affected every year by this climate (Bălteanu & Popovici, 2010). Among various types of soils, in dry climates sandy soils are more susceptible to loss of fertility, erosion and desertification (Dregne, 1986).

Naturally lavender occur on rocky, calcareous and poor soils, but different studies have revealed its plasticity on different soils (Zhelijazkov & Nielsen, 1996; Kotsiris et al., 2012; Najar et al., 2019; Vidican et al., 2023) and positive reaction to fertilizers (Biesiada et al., 2008; Mirrabi et al., 2014; Camen et al., 2016; Chrysargyris et al., 2017; Skoufogianni et al., 2017; Komnenić et al., 2020; Kvaternjak et al., 2020; Minev, 2020; Mavandi et al., 2021; Pryvedeniuk et al., 2023). However, in dry climates, soil recovery from negative changes is more problematic and lower than in wet climates (Squires & Glenn, 2011). Therefore, on such soils, even for drought resistant crops adapted to low fertility soils such as lavender, organic fertilization is essential for their resilience. Soils rich in organic matter tend to preserve better the water and consequently make more efficient use of rainfall or irrigation by crops (Bot & Benites, 2005).

As other plants from Mediterranean region, lavender grows on soil with low content of organic matter. Despite of this fact, organic fertilization proved to increase not only the flower yield (Seidler-Łożykowska et al., 2014; Skoufogianni et al., 2017; Komnenić et al., 2020), but also have influence on essential oil content (Mavandi et al., 2021; Dobreva et al., 2023).

In the current study, a field experiment was set up in south-eastern Romania on a sandy soil, in order to reveal the effects of organic fertilization versus mineral fertilization expressed by growth, flower initiation, flowering period and yield of young lavender plants.

MATERIALS AND METHODS

The study was conducted an experimental field situated in Rădulești village, south-eastern Romania (44°46'N, 26°21'E, 81 m a.s.l.) during 2021-2023. In this area, the climate is dry continental, with dry and hot summers, followed by mild winters with little precipitations.

Two years old rooted cuttings of *Lavandula angustifolia* 'Sevtopolis' were planted in November 2021, at 1 m inter-row distance and 0.50 m within row.

The soil of the experimental field was sandy, with a pH of 7.6 and both low in essential nutrients and humus (Table 1).

Table 1. Soil characteristics before treatments (0-30 cm)

Parameter	Data
Soil texture	Sandy loam
Sand (%)	64.4
Clay (%)	16.9
Silt (%)	18.7
pH (1:2.5)	7.6
EC (dS/m)	0.14
Organic carbon (%)	0.31
Humus (%)	1.98
N (%)	0.02
P (ppm)	7.0
K (ppm)	123

Lavender plants were differentially fertilized, organic and mineral, before vegetation in April. Organic fertilization with yard waste compost was achieved using 60 t ha^{-1}. The compost was obtained within the University of Agronomical Science and Veterinary Medicine Bucharest, using leaves, grass clippings, weeds, woody pruning and waste from vegetable and flower plants collected from the campus park, fields and greenhouses. Mineral fertilization was applied using 400 kg/ha YaraMila Cropcare, which contains 11-11-21 NPK and micronutrients. In the study, an unfertilized control plot was used for comparison.

Plants were irrigated weekly during vegetation period. Weeds were controlled manually when necessary. No pest treatments were applied during study. No plant protection was applied in winter. After the first growing season, plants remain to be observed without any fertilization. Lavender plants were trimmed to 15 cm in the first season, before fertilization and to 25 cm in the second season of growth, in April.

Harvesting of inflorescences was initiated gradually as they reached the full flowering stage, in order to establish the natural duration of flowering.

The effect of organic and mineral fertilization on plants growth, flowering and dormancy were analysed using one-way ANOVA test. The statistical differences between means were estimated with the Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSIONS

Lavender plants were responsive at fertilization type (Table 2). In the first year of growth, mineral fertilized plants grew significantly less ($P>0.05$) than those organic fertilized.

Table 2. Growth parameters of *Lavandula angustifolia* 'Sevtopolis' under organic and mineral fertilization

Fertilization type	Plant height (cm)		Plant diameter (cm)	
	Year 2022	Year 2023	Year 2022	Year 2023
Organic	32.0 a	29.5 a	26.5 a	34.9 a
Mineral	28.0 b	29.3 a	23.8 b	35.4 a
Unfertilized	33.2 a	27.6 a	27.6 a	37.4 a
LSD	*1.89*	*1.71*	*2.40*	*3.58*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

Mineral fertilizers tend to release nutrients quickly compared to organic fertilizers. Some researchers showed that growth of lavender plants depends on the level of nitrogen and phosphorus in the soil. Yasemin et al. (2017) found that the highest level of nitrogen (800 mg L^{-1} N) applied to young *Lavandula dentata* plants in pots had a significant negative effect on both their height and root length. Chrysargyris et al. (2016) showed that lower concentration of phosphorus had a significant negative effect on the growth of lavender plants (*Lavandula angustifolia*) under hydroponic conditions.

In our study, organic fertilized plants (yard waste compost) were not significantly different in both height or diameter from unfertilized plants. In yard waste composts, the nitrogen content varies with the components that are mixed. Michel et al. (1993) showed that nitrogen losses by volatilization during composting when grass ratio increases in the mix. In an experiment conducted by Hue et al. (1994), yard waste compost was found to increase phosphorus availability to plants.

In the second season (year 2023), when lavender plants grew with residual fertilizers, they were almost the same in height and diameter.

The fertilization type did not significantly affect the occurrence and duration of phenological stages in the first phases of plants development (Table 3).

Table 3. Phenological response of lavender plants at organic and mineral fertilization

Fertilization type	Phenological stages (days)			
	Start of growth to bud initiation	Bud initiation to flowering	Last harvest to second flowering	Last harvest to dormancy
Organic	30.4 a	11.8 a	75.7 a	55.7 a
Mineral	29.8 a	12.1 a	90.4 b	46.7 b
Unfertilized	30.2 a	11.8 a	74.7 a	56.2 a
LSD	*0.92*	*1.03*	*1.58*	*4.66*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

All lavender plants started their vegetation in the third decade of April, when the maximum air temperature was around 22-25°C. Floral bud initiation was noted on average after 30 days for all fertilization types. No significant differences were observed among variants in the number of days from bud initiation to flowering, on average 11.8 days for organic fertilized plants and 12.1 days for mineral fertilized ones.

Regardless fertilization type, all lavender plants had a second wave of flowering in the fall. However, mineral fertilization significantly delayed the second flowering of the plants. Compared to organic fertilization or unfertilized plants, mineral fertilized lavender opened their flowers on average of 90.4 days after the last harvest of the first blooming wave (almost two weeks later). Delay of flowering in lavender can be caused by high nitrogen content of the soil (Matysiak and Nogowska, 2016), which stimulate the vegetative growth.

Significant differences were also remarked for dormancy in mineral fertilized plants. After the last harvest of the second flowering, these entered into dormancy 10 days before both the unfertilized and organic fertilized plants.

Organic fertilized plants followed almost the same trend as unfertilized plants, with no significant differences in the phenological stages.

First flowering wave started in June for all plants independently of the fertilization type. Also, all plants recorded a second wave of flowering in the fall. Lavender 'Sevtopolis' has been reported as a late flowering cultivar compared to other Bulgarian cultivars (Stanev & Angelova, 2023) and the oil's quality from the second flowering is perfectly suitable for perfumery use (Zhekova & Nedkov, 2011). In our study, this cultivar reached maximum flowering in mid-June (Figure 1) independent of fertilization type. Its earliness can be explained by the higher temperatures recorded in the last decade during summer months, in south-east of Romania.

In the second flowering wave, organic fertilized plants and those unfertilized began to flower in mid-September. Mineral fertilized lavender started to flower two weeks later, in the first decade of October. However, the flowering period was not significantly influenced by the type of fertilization neither in the first, nor in the second flowering wave (Table 4).

The fertilization type had a significant impact on floral stems produced by the plants. The best flowering was recorded on unfertilized plants, from which 78.8 stems/plant were harvested during summer. At this time, organic fertilized plants produced a significantly lower number of 37.5 floral stems/plant. Various studies have shown that organic fertilizers generally increase the number of floral stems on the plant (Seidler-Łożykowska et al., 2014; Komnenić et al., 2020). However, some other research has revealed that lavender plants can react differently to composts and their dose (El-Ghadban et al., 2008; Mavandi et al., 2021; Shoeip et al., 2022). In our study, yard waste compost reduced flower production in the first flowering wave, but not in the second or subsequent year of studies.

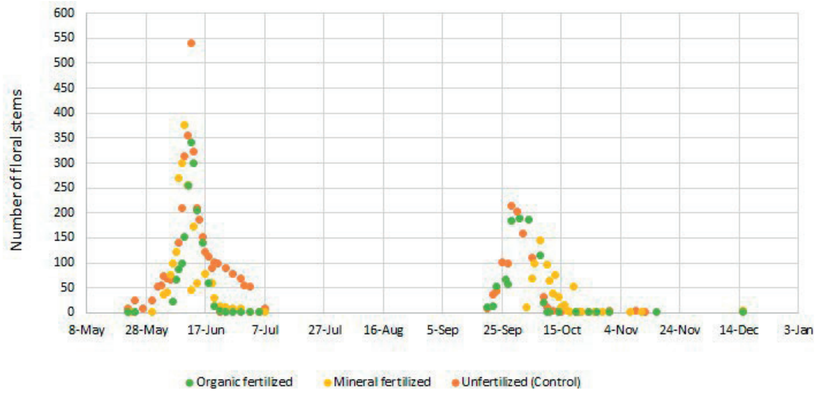

Figure 1. Flowering dynamics of *Lavandula angustifolia* 'Sevtopolis' under organic and mineral fertilization

Table 4. Flowering parameters of *Lavandula angustifolia* 'Sevtopolis' under organic and mineral fertilization

Fertilization type	Flowering duration (days)		Number of floral stems/plant		Stems length (cm)		Inflorescence length (cm)	
	1st wave	2nd wave	1st wave	2nd wave	1st wave	2nd wave	1st wave	2nd wave
Organic	36.0 a	46.4 a	37.5 a	10.5 b	19.2 a	13.0 b	8.2 b	8.0 b
Mineral	34.2 a	41.9 a	44.1 b	8.5 a	19.0 a	11.3 a	7.4 a	5.0 a
Unfertilized	34.7 a	38.4 a	78.8 c	12.0 c	21.7 b	11.5 a	9.0 c	5.5 a
LSD	*4.62*	*23.7*	*6.16*	*1.42*	*1.65*	*1.03*	*0.78*	*1.02*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

All lavender plants, fertilized and unfertilized, produced fewer floral stems during autumn compared to summer. In autumn, only 12 floral stems/plant were harvested from unfertilized plants, but significantly more than mineral fertilized ones (8.5 floral stems/plant).

Various studies have reported a significant effect of macronutrients on the number of floral stems of *Lavandula* species. Potassium applied in the form of KCl has been shown to have a positive influence on the number of inflorescences in *Lavandula dentata* (Peçanha et al., 2023). Pryvedeniuk et al. (2023) reported in *Lavandula angustifolia* an increase of inflorescences yield by combining mineral fertilization with foliar application of nitrogen by 8.1%, phosphorus by 4.4%, potassium by 7.4% and NPK by 9.6%.

Floral stems had the same length in both types of fertilization, organic and mineral, but only for the first flowering wave. However, these were shorter than the floral stems of unfertilized plants. In the second flowering wave, organic fertilized plants had significantly longer floral stems of 13.0 cm compared to mineral fertilized or unfertilized plants, of 11.3 cm and 11.5 cm, respectively.

Organic fertilization stimulated the development of long inflorescences both in summer and in autumn, of 8.2 cm and 8.0 cm, respectively. Anyway, the greatest length of inflorescences was obtained in unfertilized plants, of 9.0 cm during the summer period. In the second flowering wave, these plants formed shorter inflorescences, of 5.5 cm. Mineral fertilization negatively influenced the inflorescence length in both flowering periods. These were significantly shorter than those formed by organic fertilized plants. Although with a shorter length, the inflorescences of mineral fertilized plants were denser and heavier. Therefore, fresh flower yield in 2022 was higher in mineral fertilized plants compared to organic fertilized ones, considering both flowering periods (Table 5). The second flowering during autumn was better for the organic fertilized plants, which recorded a yield of 86.1 $kg.ha^{-1}$. Anyway, it should be noted that the unfertilized plants achieved an almost double production of fresh flowers compared to both organic or mineral fertilized plants.

Table 5. Fresh flower yield under organic and mineral fertilization

Fertilization type	Yield in 2022 (kg.ha^{-1})		Yield in 2023 (kg.ha^{-1})	
	1st wave	2nd wave	1st wave	2nd wave
Organic	311.25	86.1	2990.4	644.43
Mineral	313.11	44.2	3510.9	662.11
Unfertilized	693.44	67.2	2870.0	640.08

In 2023, the highest yield was obtained in mineral fertilized plants, of 3510.9 kg.ha^{-1} in the summer and 662.11 kg.ha^{-1} in the fall. Organic fertilized plants also exceeded the yield of unfertilized plants, both at the first harvest and the second harvest.

Organic and mineral fertilization did not improve the yield in the first year when these were applied, but in the second year. These results indicate a delayed reaction of plants to fertilization in the tested conditions and can probably be explained by the priority in using resources for roots development over the aerial part, as a result of the growing conditions on sandy soil and hot summer.

CONCLUSIONS

Yard waste compost proved to be a fertilizer that can be used in lavender crops, but not superior to mineral fertilizer in terms of yield. The positive effects of this organic fertilizer were observed only one year after application, when the yield exceeded that of unfertilized plants. Further research is needed to establish the amount and frequency of using this green compost in order to obtain comparative or superior benefits to mineral fertilizers.

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ANATOMICAL RESEARCH OF THE VEGETATIVE BODY OF *IMPATIENS GLANDULIFERA*, AN ORNAMENTAL PLANT THAT HAS BECOME INVASIVE

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Abstract

Ornamental horticulture is a significant contributor to the introduction of invasive plant species, such as Impatiens glandulifera. Native to the temperate and humid regions of Asia, particularly the Himalaya Mountains, the species was first reported in Europe in 1839, introduced for ornamental and nectar-producing purposes. Its presence in Romania dates since 1882. Over the past few decades, the species' invasive nature has led to its inclusion in the List of Invasive Alien Species of Union concern. The existing studies have systematically examined the plant's morphology, habitat requirements, ecology, life cycle, and genetic aspects, emphasizing the traits associated with its high invasive potential. The species exhibits a preference for moist to wet and nutrient-rich soils, commonly found in river valleys and associated riparian habitats. Our research, based on samples collected from Romania, aims to identify and detail the structural features of the vegetative body of Impatiens glandulifera that contribute to its adaptive ability to specific environmental conditions. The anatomical investigations revealed a secondary root and stem structure, enhancing this annual species' robustness and resistance in plant communities.

Key words: *alien plants, structural adaptations, wet environment, environmental requirements.*

INTRODUCTION

The increase of invasive alien species is a growing concern, with their spread posing significant threats to ecosystems worldwide. These invaders can affect native species by altering their abundance, diversity, and even driving them towards extinction. Furthermore, the disruptive effects of invasive species extend to ecosystem functioning, with consequences that are expected to increase in the future (Pyšek et al., 2020).

The introduction of invasive species into new regions can result from various human activities, either deliberate or unintentional (Pyšek et al., 2020; Pyšek et al., 2004, Richardson et al., 2000).

The horticultural industry, widely recognized as one of the main pathways for introducing invasive species worldwide, plays a significant role in this (Bayón & Vilà, 2019).

Invasive alien species are defined as non-native species that establish themselves in ecosystems or natural habitats, posing threats to native biological diversity. Such alien species, also referred to as allochthone, non-indigenous, or exotic species, have the ability to disperse or reproduce beyond their native range (IUCN, 2000).

Impatiens glandulifera Royle, a species of the Balsaminaceae family (Clements et al., 2008; Kraehmer, 2013; Caris et al., 2006; Helsen et al., 2021), is native to northeastern Pakistan, northwestern India, and western Nepal, thriving in temperate climates, between 2000 and 4000 m above sea level (Beerling & Perrins, 1993; Helsen et al., 2021; CABI, 2023).

In Europe, the species was first reported from England in 1839 (Drescher & Prots, 2003). Within approximately four decades, it became naturalized, spreading across nearly all European countries (Pyšek et al., 1995; Helsen et al., 2021). Currently, the species is distributed in 34 European countries, including Romania (CABI, 2023).

In Romania, *Impatiens glandulifera* was documented for the first time in 1882, from Sibiu (Balog, 2008; Sîrbu & Oprea, 2011). According to the literature, the species is widely distributed throughout the country, excluding Banat, Oltenia and Dobrogea regions (Oprea, 2005; Sîrbu & Oprea, 2011; Dumitraşcu et al., 2014; Anastasiu et al., 2020). Notably, it has also been reported from natural or national parks (Dumitraşcu et al., 2014; Anastasiu et al., 2022). The species' rapid growth rate is highlighted by its ability to reach up to 1.3 meters in height within 72 days of germination (Perrins et al., 1993; Helsen et al., 2021). *Impatiens glandulifera* thrives in riparian habitats, including moist forests, swamps, stream sides, ditches, in full light, or partial shade (Balogh, 2008; Sîrbu & Oprea, 2011; Anastasiu et al., 2019). It commonly inhabits hilly and mountainous regions (Sîrbu & Oprea, 2011; Anastasiu et al., 2005; Anastasiu et al., 2019). This species can impede the growth and development of native plants and can spread along watercourses, thus constituting a danger for nature conservation (Pyšek & Prach, 1995; Anastasiu et al., 2019).

Several factors contribute to the success of the invasion of *Impatiens glandulifera*. One such factor is the species' ability to grow up to 4 meters in height, being the tallest annual plant in Europe, making it the strongest competitor against native grass species. Additionally, the species' prolific seed production, with a single plant capable of producing up to 2500 seeds dispersed over distances of up to 7 meters, further amplifies its invasive potential (Balogh, 2008; Ab Razak et al., 2023). Moreover, the seeds can persist as a seed bank for at least 18 months (Mumford, 1990).

Despite extensive research on the morphology, habitat requirements, and ecology of *Impatiens glandulifera*, information regarding the structural characteristics of its vegetative organs remains scarce. Thus, in this paper we aim to analyse the vegetative body's structure of *Impatiens glandulifera* to identify the histo-anatomical features that highlight its adaptability to diverse environmental conditions and habitats.

MATERIAL AND METHODS

Study species. *Impatiens glandulifera* Royle [*I. royle* Walp.] (Fam. Balsaminaceae), commonly known as Himalayan balsam, is an adventitious and invasive plant (Ciocârlan, 2009; Sîrbu & Oprea, 2011). Initially introduced as an ornamental plant, the species has escaped cultivation and became naturalized (Sîrbu & Oprea, 2011; Georgescu & Luchian, 2023).

Impatiens glandulifera is an annual, glabrous plant that can reach heights up to 2-2.5 meters (Beerling & Perring, 1993; Anastasiu & Negrean, 2007; Pioarca-Ciocanea et al., 2020). The stems are hollow, and the nodes are thickened (Balogh, 2008; Helsen et al., 2021).

The primary roots are cylindrical with a diameter of 2-3 mm, while the adventitious roots are conical and can grow up to 15-20 cm long. These roots can reach into the soil to depths of 10-50 cm (Clements et al., 2008; Balogh, 2008; Helsen et al., 2021).

The leaves are petiolate, arranged in whorls of three, ovate-lanceolate in shape, with serrate margins. Dark red glands can be observed at the nodes of the petiole (Săvulescu, 1958; Pioarca-Ciocanea et al., 2020).

The flowers are formed in the axils of the upper leaves, arranged in erect racemes, with 2-14 large flowers that can reach up to 3-3.5 cm and are lilac pink in color. The flowers are zygomorphic (Săvulescu, 1958; Pioarca-Ciocanea et al., 2020).

The fruit is a capsule that twists into a spiral when ripe, facilitating the dispersal of seeds over long distances (Săvulescu, 1958; Balogh, 2008; Anastasiu & Negrean, 2007; Anastasiu et al., 2019; Pioarca-Ciocanea et al., 2020).

The seeds are pale grey-brown, turning black when mature. They are oval-shaped, measuring 3-4(7) mm in length, 2-4.8 mm in width, and 1.5-2 mm in diameter (Balogh, 2008; Helmisaari, 2010).

Methodology. Plant material, consisting of specimens measuring 1.5-2 m in height, was collected on 22nd of July 2023 from the DJ101P 173, Răgman (45.143111°N, 25.696358°E). The collected biological material was fixed in 70% ethyl alcohol.

Cross-sections for structural analyses were performed through adventitious roots, principal stem in the basal, median, and tip areas, as well

as through the petiole, and leaf. The cross-sections were further processed using the double coloration technique (Iodine Green and Carmine Alum). Starch was highlighted by soaking the sections in IIK solution (Şerbănescu-Jitariu et al., 1983).
After analyzing the resulting microscope slides, they were photographed with the optical microscope.

RESULTS AND DISCUSSIONS

Root Structure: The root system of *Impatiens glandulifera* comprises a cylindrical primary root, along with numerous lateral and adventitious roots (Helsen et al., 2021). The analyzed adventitious roots exhibit a secondary structure resulting from the activity of the secondary meristems, cambium and phellogen. The phellogen produces several layers of cork outward and phelloderm inward. The vascular cambium generates secondary phloem externally and secondary xylem internally.
In cross-section, the adventitious root displays a circular shape and the following distinct anatomical regions: the epidermis (rhizodermis), as the outermost layer featuring root hairs, a cortex, and a central vascular cylinder (Figure 1 - A).
The cortex of the adventitious root contains an aerenchyma formed by isodiametric cells with large gaps (Figure 1 - A, B). The endodermis, composed of tangentially elongated cells with Casparian strips, forms the innermost layer of the cortex (Figure 1 - B). Notably, some cortex cells contain raphides.
The pericycle, consisting of parenchyma cells of varying sizes, forms the outermost layer of the vascular cylinder, positioned beneath the endodermis (Figure 1 - B).
Within the vascular cylinder, the secondary phloem and xylem have a circular disposition, with a reduced phloem, located outward. In the cross-section of the adventitious root, 16-20 vascular bundles are arranged in a circle (Figure 1 - A). The xylem comprises wood vessels of various diameters, along with lignified wood parenchyma, and wood fibers.
At the center of the vascular cylinder there is a parenchymatous pith, formed of large, polygonal cells with small intercellular spaces (Figure 1 - A, B), some of which contain raphides.

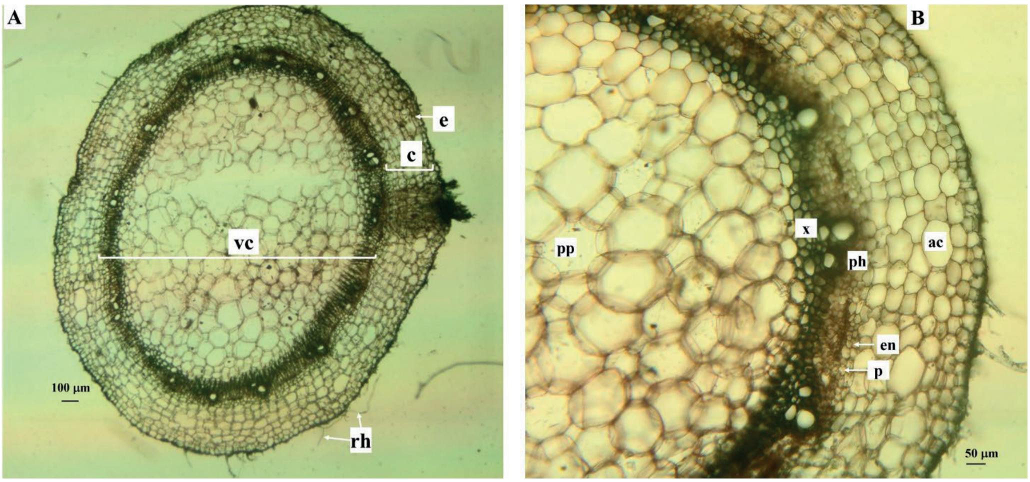

Figure 1. Cross-sections of *Impatiens glandulifera* adventitious roots, highlighting the the different anatomical areas (A - overview, B - detail) (colorants: Iodine Green and Carmine Alum): ac - aerenchyma, c - cortex, e - epidermis, en - endodermis, p - pericycle, ph - phloem, pp - parenchymatous pith, rh - root hairs, vc - vascular cylinder, x - xylem

Stem structure: In cross-sections performed at three levels of the stem (lower, median, and upper third), the contour shifts from nearly circular in the lower third to slightly ridged in the median third and prominently ridged in the upper third, where 6 ridges differentiate (Figure 2 - A, B, C).
The stem has a secondary structure resulting mainly from the activity of the vascular cambium. At all three levels analyzed, the stem

displays the following anatomical areas: epidermis, cortex, and vascular cylinder with a large medullary lacuna in the center (Figure 2 - A, B, C).

The epidermis is unstratified, formed of small isodiametric cells covered by a thin cuticle. The cortex comprises parenchymatous polygonal cells that increase in size towards the vascular cylinder. The number of cortex layers increases from 5-6 in the lower third to 6-8 in the upper third and 10-12 in the ridge area (Figure 2 - D, E, F).

Beneath the epidermis, the outer cortex comprises an angular collenchyma. The number of layers of this mechanical tissue increases from 2-3 in the lower third of the stem, to 3-5 in the upper third. The innermost layer of the cortex accumulates starch and differentiates an amyliferous sheath (Figure 2 - F, G).

The pericycle, consisting of small parenchyma cells, forms the outermost layer of the vascular cylinder and it is positioned beneath the amyliferous sheath (Figure 2 - D, E, F). The secondary vascular tissues are arranged concentrically, with a thin area of phloem (outward) and a thick area of xylem in the lower third of the stem. In the secondary xylem, the predominant wood fibres (libriform) have a radial arrangement (Figure 2 - D).

Secondary xylem development is more pronounced in the lower third of the stem, with an almost concentric arrangement, contrasting with the fascicular organization observed in the median and upper thirds of the stem (Figure 2 - D, E, F). In the median and upper third, at the ridges level and between these areas, vascular bundles are distinguished, concentrically arranged, with 2-3 in the median third and 3-4 in the upper third of the stem (Figure 2 - E, F).

Needle-like crystals composed of calcium oxalate (raphides) were observed in the cells of the cortex and vascular cylinder (Figure 2 - G).

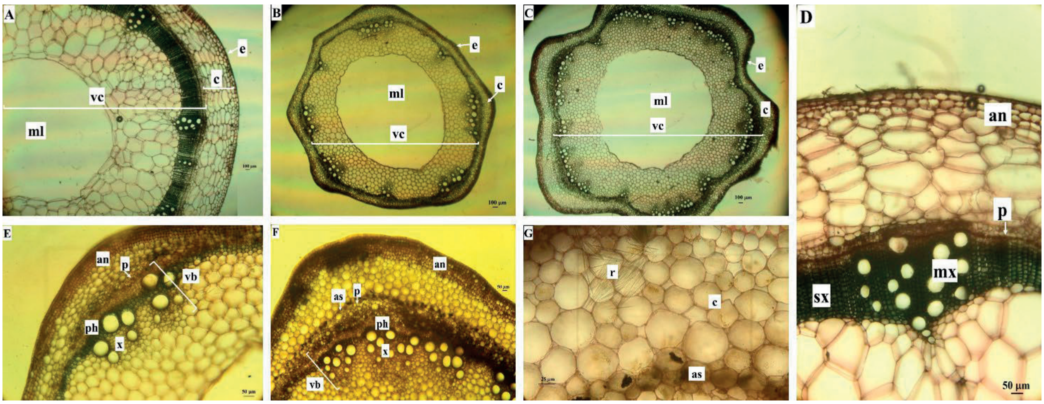

Figure 2. Cross-sections of *Impatiens glandulifera* stem (A, D - lower third; B, E - median third; C, F, G - upper third) (colorants: Iodine Green and Carmine Alum, IIK): an - angular collenchyma, as - amyliferous sheath, c - cortex, e - epidermis, ml - medullary lacuna, mx - metaxylem, p - pericycle, ph - phloem, r - raphides, sx - secondary xylem, vc - vascular cylinder, x - xylem

The lamina of *Impatiens glandulifera* exhibits a bifacial dorsiventral structure. In cross-sections, the lamina displays prominent features on both faces around the midbrid, with an elongated contour on the adaxial face (Figure 3 - A).

The upper and lower epidermis, corresponding to the adaxial and abaxial faces, consist of isodiametric cells covered by a thin cuticle.

At the midbrid level, vascular tissues are organized into a closed collateral vascular bundle arranged in an open arch (Figure 3 - A). The phloem is positioned towards the abaxial face and is bordered by an amyliferous sheath. The vascular bundle is supported by an angular collenchyma located beneath both epidermis around the midbrid (Figure 3 - A).

Between the angular collenchyma and the vascular bundle, several layers of parenchymatic cells with cellulosic walls and small to larger intercellular spaces can be distinguished (Figure 3 - A).

The mesophyll comprises one layer of palisade cells beneath the upper epidermis, a layer of collector cells beneath the palisade tissue, and a

lacunose tissue consisting of 5 to 6 layers of isodiametric cells with large gaps, situated above the lower epidermis (Figure 3 - B).
The leaf is amphystomatic, with stomata located at approximately the same level as the epidermal cells (Figure 3 - B). Additionally, certain parenchymatic cells within the lamina contain raphides of calcium oxalate.

The petiole of *Impatiens glandulifera* exhibits a fistulous structure with a polygonal external outline, featuring two lateral elongations (Figure 3 - C). Both, the upper and lower epidermis consist of a single layer of isodiametric cells, covered by a thin cuticle (Figure 3 - C). The petiole is supported by a mechanical tissue comprised of 4-5 layers of continuous angular collenchyma situated beneath the entire surface of epidermis (Figure 3 - D).
The vascular tissues are organized in several vascular collateral bundles, forming a semicircular pattern towards the lower epidermis (Figure 3 - C). Adjacent to the phloem, a distinct amyliferous sheath was observed (Figure 3 - D). The vascular bundles are surrounded by a lacunose tissue composed of isodiametric cells with small to large gaps, accompanied by angular collenchyma (Figure 3 - D). Towards the adaxial face, this tissue defines a lacuna (Figure 3 - C).

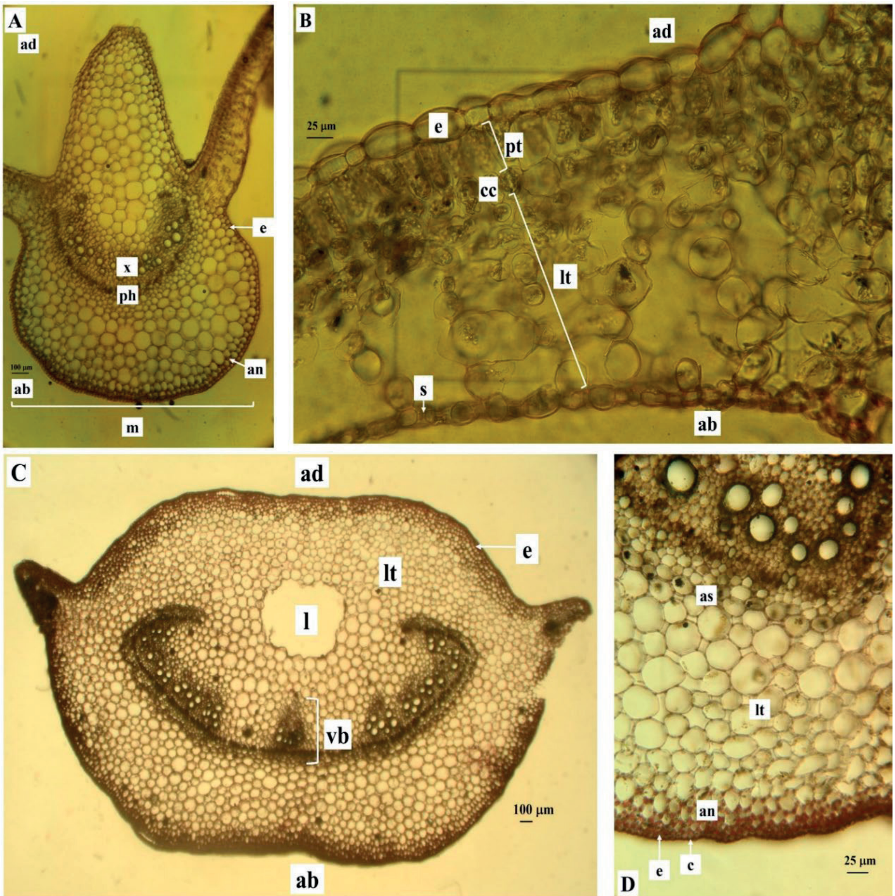

Figure 3. Cross-sections of *Impatiens glandulifera* leaf (A - lamina, highlighting the structure of the midrib; B - lamina, highlighting the mesophyll structure; C, D - petiole), (colorants: Iodine Green and Carmine Alum, IIK): ab - abaxial face, ad - adaxial face, an - angular collenchyma, as - amyliferous sheath, c - cuticle, cc - collector cells, e - epidermis, l - lacuna, lt - lacunose tissue, m - midbrid, ph - phloem, pt - palisade cell, s - stomata, vb - vascular bundle, x - xylem

The anatomical analysis of *Impatiens glandulifera*'s vegetative body has revealed a secondary structure of both root and stem systems. These characteristics, associated with a structural robustness and rapid growth of the corm, provide notable advantages to this annual species, ensuring its competitiveness over native flora within its habitat (Wang et al., 2022; Dumitrașcu et al., 2023).

Additionally, similar features, such as a well-developed secondary structure of roots and stems, have been observed in other annual alien species like *Symphyotrichum cilliatum* and *S. squamatum* (Sârbu & Smarandache, 2015; Dumitrașcu et al., 2023).

Successful plant invasion relies upon efficient nutrient absorbtion and utilization, as well as adaptation to diverse environmental conditions. This often includes effective competition for resources such as water and nutrients. Therefore, plants with strong competitive abilities often possess advanced root systems (Wang et al., 2022).

The root system of *Impatiens glandulifera*, characterized by a wide stem base acting as a rigid anchor point, numerous adventitious roots with fleshy composition and strengthening elements increase the mechanical efficiency of the roots for anchorage (Ennos et al., 1993). Additionally, the presence of aeriferous tissue from the adventitious roots, enables it to thrive in wet environments, indicating its adaptation to such conditions. These structural adaptations, along with the well-developed adventitious roots with secondary structure, allow *Impatiens glandulifera* to outcompete native species by enhancing its anchorage, increasing resistance to pullout and bending forces, and ensuring stable growth within its habitat (Ennos et al., 1993).

The stem of *Impatiens glandulifera* exhibits structural adaptations that support its invasive characteristics, in line with findings regarding plant growth goals, adaptation to heterogeneous environments, and fruit production observed in invasive species (Wang et al., 2022). Similar to other invasive plants, *Impatiens glandulifera* demonstrates robust stem architecture, allowing it to support the inflorescences produced mainly on the upper parts of the stem. This structural strength is essential for bearing the load of the inflorescences and ensuring efficient nutrient transport, as highlighted by studies on other invasive plants such as *Solidago canadensis* (Wang et al., 2022). The presence of highly differentiated secondary xylem in *Impatiens glandulifera's* stem further enhances its support function, similar to the advanced supporting fiber structures found in other invasive plants (Sârbu & Smarandache, 2015; Wang et al., 2022; Dumitrașcu et al., 2023). These structural adaptations contribute to *Impatiens glandulifera*'s invasive success by facilitating its growth, reproduction, and competitive ability in diverse ecological environments.

Furthermore, the leaf structure of *Impatiens glandulifera* exhibits adaptations for effective sunlight absorption and nutrient transport. Its bifacial leaves with a well-developed layer of palisade tissue ensure efficient sunlight absorption, while the presence of thick leaf veins, as well as the presence of the collector tissue, enhances nutrient transport efficiency (Wang et al., 2022).

The presence of angular collenchyma in above-ground organs provides considerable strength and elasticity, allowing the plant to bend without breakage (Carrillo-López & Yahia, 2019). Moreover, the presence of calcium oxalate raphides in all examined vegetative organs most likely enhances their resistance to environmental stress factors.

These structural adaptations observed in *Impatiens glandulifera* and other invasive plant species, contribute to their invasive success by enabling effective colonization and dominance in new habitats. These findings emphasize the importance of understanding the anatomical basis of plant invasiveness for effective management and control strategies.

CONCLUSIONS

Overall, *Impatiens glandulifera* exhibits a robust anatomical structure characterized by secondary growth, which confers competitive advantages in resource acquisition and growth rates compared to native plant species. The presence of the aeriferous tissue in adventitious roots, enables adaptation to diverse soil conditions, especially wet environments. Additionally, the presence of angular collenchyma in above-ground organs provides mechanical support and flexibility, facilitating the plant's ability to withstand environmental stresses.

While this invazive plant has been extensively studied, the literature contains insuficient data regarding the structure of *Impatiens glandulifera's* vegetative body. Our detailed analysis enhances the understanding of the adaptive capacity *Impatiens glandulifera* to environmental conditions and underscores the importance of considering anatomical traits in invasive plant species management strategies.

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EVALUATION OF SOME HYACINTH CULTIVARS UNDER OPEN FIELD CONDITIONS

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Abstract

The hyacinth is one of the most important spring-flowering bulbous plants, extremely appreciated for its beautiful and fragrant flowers in attractive colours, which is used in flowerbeds, borders, small groups, mass plantings of single variety or colour for a greater visual impact in parks and gardens, patio containers, and as cut flowers in bouquets and floral arrangements. It is also grown in pots and forced to bloom indoors in late winter. The aim of the study was to evaluate the growth and flowering characteristics of nine hyacinth cultivars grown in open field conditions. The results revealed that the maximum plant height (31.25 cm), length of the leaves (29.38 cm) and inflorescence length (14.33 cm) were recorded in 'Woodstock'. Among the studied cultivars, 'Blue Jacket' produced the highest number of flowers in inflorescence (44.36), 'Purple Pride' recorded the minimum number of days from planting to flowering (146.16 days) and 'Fondant' had the longest flowering duration (15.12 days).

***Key words**: Hyacinthus orientalis, cultivars, growth, flowering*

INTRODUCTION

Hyacinthus orientalis L. is a perennial bulbous species in the Liliaceae family with tunicate bulbs that produce an upright, thick and fleshy, succulent flower stem, measuring 20-30 cm in height. The basal leaves are linear, narrow, glossy and deep green. The inflorescence is a more or less dense raceme composed of numerous small, single or double, waxy flowers in various shades of white, pink, red, yellow, orange, blue, and purple, each having a six-lobed, tubular perianth. The bright colour of the flowers is attributed to anthocyanins, the most common plant pigments identified and isolated in the flowers of various hyacinth cultivars, with some differences in composition (Hosokawa et al., 1996; Hosokawa, 1999; Mulholland et al., 2013; Tao et al., 2015). Xie & Wu (2017) reported that the morphology, colour, flowering duration, and quality of flowers can be modulated by inoculation with arbuscular mycorrhizal fungi. The flowers emit an intense and sweet characteristic fragrance that attracts bees. Hyacinths bloom in early to mid-spring (March-April), for two to three weeks, depending on the cultivar and the local climate, at the same time with other spring-flowering plants such as pansies, alpine forget-me-not, common daisies, primroses, daffodils, and some varieties of tulips, etc. They prefer light, sandy, and sandy-loam soils, with the proper pH, moderately fertile and well-drained. Therefore, heavy, compact soils or those difficult to drain after abundant rains should be avoided. It grows and blooms best in full sun, but can also tolerate partial shade. Temperature has an essential role in controlling plant growth and flowering in geophytes. The optimal temperature for floral induction and early stages of organogenesis in hyacinths is between 17-25ºC, followed by a period of low temperatures (4-9°C), crucial for floral stalk development and anthesis in spring (Khodorova & Boitel-Conti, 2013).

Hyacinths are vegetatively propagated through bulbs, which can be globose to ovate and covered with tunics of different colours such as white, cream, silvery-purple, or dark purple, depending on the cultivar grown. These bulbs, which store water and nutrients, are planted in the autumn, typically from late September to November, depending on the location, to bloom the following spring.

The hyacinth bulbs naturally produce very few bulblets. Therefore, various artificial methods have been developed to stimulate bulblets formation at the base of the mature bulb and increase the multiplication rate. Techniques of propagation involving cuts, either in cross or radial through the basal plate of a dormant bulb (scoring), or scooping out the central portion of the basal plate with a small sterilized knife, while leaving the outer edge intact (scooping), are used to produce multiple new bulblets in a shorter time. Small bulblets will form along the cut surfaces, which are then planted separately from the bulbs and require three to four years to reach the normal size and start blooming. In the scooping method was obtained the highest number of bulblets per bulb, while the diameter, height and weight of the bulblets registered maximum values when the scoring technique was used (Zahraei Basir, 2022). Lexow & Bobadilla (2013) reported that by the scooping method was produced a greater number of bulblets than by cross-cutting in 'Splendid Cornelia', 'Carnegie', and 'Atlantic' hyacinth cultivars. Masoodi et al. (2022) concluded that the scooping technique proved efficient in propagation of the hyacinths, among the studied cultivars, the best results being obtained at 'Yellowstone' cultivar. The hyacinth bulbs do not sprout if planted immediately after harvesting, even under favorable growing conditions. A cold treatment period of several months is necessary to release their dormancy and accelerate the leaf emergence (Gude & Dijkema,1992; Karjee & Mahapatra, 2019).

Conventional propagation of hyacinths is slow, therefore the micropropagation is an efficient alternative method for rapid production of a superior planting material. *In vitro* propagation techniques are used to meet the market demands for some economically valuable ornamental geophyte species (Çiğ & Başdoğan, 2015). These techniques are based on the plantlet formation and bulblet regeneration, using various types of explants such as bulb scale segments and leaves, that are the most widely used (Yi et al., 2002; Lee et al., 2007; Salehzadeh et al., 2008; Sun et al., 2010; Gheisari & Miri, 2017; El-Naggar et al., 2023), flower buds (Wen-liang et al., 1999), peduncle (Ziv & Lilien-Kipnis, 2000), fruits containing immature zygotic embryos (Kizil et al., 2016), and somatic embryogenesis (Zarnadze et al., 2019). Bulblet multiplication is influenced by various factors, including light, temperature, humidity, the size and type of the explants used, plant growth regulators, carbohydrates, and the type of culture medium (Bach & Swiderski, 2000; Chung et al., 2006; Li et al., 2023). Some hyacinth cultivars can also be propagated by leaf cuttings, and after introduction into the rooting medium, adventitious roots and new bulbils form at their basal part (Krause, 1980).

Hyacinths are cultivated as ornamental plants in parks and gardens, and can be used in monochromatic or mixed-colour combinations in flowerbeds, mixed borders, mass plantings for a maximum visual impact, near building entry areas, patios, along walkways, or in outdoor pots and planters, as well as cut flowers in bouquets and floral arrangements (Seyidoğlu et al., 2009). Bulbs can be forced to grow and bloom indoors earlier than in the garden. Prepared bulbs that have 12-14 weeks of pre-cooling at 9°C, are utilized for forcing either in soil in pots (Śmigielska et al., 2014), or in water using special glass vases and in hydroponic system *(*Krzymińska, 2008; Çiğ & Koçak, 2019; Wu et al., 2021*)*. A bulb storage temperature of 5 or 10°C is recommended before planting in pots to achieve rapid and quality flowering (Nalousi et al., 2018). For the growth of hyacinth in pots, Addai (2011) recommended the application of $(NH_4)_2SO_4$ to enhance plant growth and bulb yield, and Na_2HPO_4 to improve flower quality, both nutrients at a rate of 60-90 mM. Controlling the height of potted plants is important to prevent stem elongation and curvature, especially when bearing a heavy inflorescence, and this is achieved through chemical methods involving growth regulators, primarily gibberellin inhibitors (Demir & Çelikel, 2013; 2019). After flowering, the bulbs are planted in the garden and bloom in the spring of the following year.

The flowers are also utilized in the perfumery industry for extraction of essential oils, which have numerous applications in cosmetics and aromatherapy (Boeriu, 2015). Brunke et al. (1994) isolated the volatiles of white hyacinths by dynamic headspace trapping and identified more than 70 constituents. Zarifikhosroshahi et al. (2022) identified 28 volatile compounds in the fresh flowers of wild hyacinths. Ethanolic extracts obtained from hyacinth flowers (Soare et al., 2012), and silver phytonanoparticles synthesized using aqueous petal extracts (Bunghez et al., 2012) have demonstrated antioxidant and antimicrobial activities. Kury et al. (2021) and Shareef & Abdul-Jalil (2023) revealed that the biologically active compounds present in the plant extracts have anticancer and immunomodulatory effects.

The experiment was conducted to study the growth and flowering behaviour of nine hyacinth cultivars under open field conditions.

MATERIALS AND METHODS

The research was carried out to evaluate the vegetative growth and flowering characteristics of nine hyacinth cultivars as 'Blue Jacket' (deep blue), 'City of Haarlem' (light yellow), 'Delft Blue' (blue), 'Fondant' (pink), 'Jan Bos' (dark pink), 'Pink Pearl' (light pink with white edges), 'Purple Pride' (purple), 'White Pearl' (white), and 'Woodstock' (deep purple) grown in open field conditions, in the Floriculture Research Area of the Faculty of Horticulture, University of Craiova, from October 2021 to April 2022. The planting material imported from the Netherlands and purchased from the local market, consisted of bulbs with a circumference of 14-15 cm and a weight ranging from 37.62 to 49.85 g. These bulbs were planted in the experimental field in October 2021, at the distances of 20 cm between rows, 15 cm between bulbs on row and a planting depth of 10 cm, in a fertile, moist and loose soil. Prior to planting in soil, the bulbs were treated with a fungicidal solution to enhance protection against diseases. The monofactorial experiment was arranged in randomized complete block design with three replications. The observations and biometric measurements were conducted at the flowering stage on various growth parameters such as plant height (measured from the surface of the soil to the apex of the inflorescence), leaf length and width, number of leaves per plant, and on flowering characteristics (inflorescence length, number of florets in the inflorescence, number of days from planting to flowering, and duration of flowering). The collected data were statistically processed using descriptive statistics and one-way analysis of variance (ANOVA) for each evaluated parameter, followed by Duncan's Multiple Range Test at $p \leq 0.05$ significance level.

RESULTS AND DISCUSSIONS

Vegetative growth parameters

The results regarding to the evaluated vegetative parameters are presented in Table 1. Significant statistical differences were observed among the hyacinth cultivars for plant height. Analysing the average values for each cultivar, it was found that the highest value for this growth parameter was recorded in 'Woodstock' (31.25 cm), followed by 'Delft Blue' (29.87 cm), while the 'Fondant' cultivar exhibited the lowest plant height (20.46 cm), indicating less vigorous growth.

The average number of leaves per plant ranged from 5.73 to 8.00, with an average of all the cultivars of 6.78 leaves. The 'City of Haarlem' cultivar recorded the maximum value, which indicated a significant number of leaves compared to other cultivars, while the minimum number of leaves per plant was observed in 'White Pearl'. Regarding the average leaf length and width, the data analysis revealed significant differences among cultivars. The highest value of leaf length was recorded at 'Woodstock' (29.38 cm), followed by 'Delft Blue' (25.76 cm), while 'City of Haarlem' exhibited the lowest leaf length (15.52 cm), and also the smallest leaf width (2.41 cm). 'Delft Blue' recorded the highest value for the leaf width (3.93 cm).

Masoodi (2022) reported significant variations among some cultivars of hyacinths for various growth and propagation traits. Kaushik et al. (2021) also reported variations attributed to cultivars for several vegetative parameters. The size of bulbs used during planting is very important, as it influences the vegetative growth of plants, making them more vigorous and robust, as well as the flower quality and the commercial value. The size of underground storage organs has a positive correlation with flowering quality (Kapczyńska, 2014; Howard & Cellinese, 2020). The increase of growth and flowering parameters in proportion to bulb size may be attributed to the amount of nutrients stored in the bulb before planting (Addai & Scott, 2011).

Table 1. Evaluation of vegetative parameters of various hyacinth cultivars studied under open field conditions

Cultivar	Plant height (cm)	Number of leaves/plant	Leaf length (cm)	Leaf width (cm)
Blue Jacket	24.16 cd	7.22 a	23.41 c	3.00 ab
City of Haarlem	23.31 d	8.00 a	15.52 f	2.41 b
Delft Blue	29.87 ab	6.33 bc	25.76 b	3.93 a
Fondant	20.46 d	6.91 ab	21.35 cd	3.00 ab
Jan Bos	26.13 c	6.66 bc	20.81 de	2.56 b
Pink Pearl	27.20 bc	6.00 bc	20.23 de	3.33 ab
Purple Pride	21.38 d	7.00 ab	18.56 e	3.53 a
White Pearl	23.92 cd	5.73 c	22.63 cd	3.25 ab
Woodstock	31.25 a	7.13 a	29.38 a	3.76 a
Mean	25.30	6.78	21.96	3.20
CV%	14.43	10.18	18.35	15.94

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan's multiple range test

Flowering parameters

The aesthetic value of the plants, but also other aspects such as the period and duration of flowering and their adaptability to various environmental conditions, are important criteria for plant selection, association and placement in urban green areas. The hyacinths are important ornamental plants used in the spring decoration of parks, as well as public and private gardens, and constitute a visual element of great attractiveness through a diverse colour palette and the multiple possibilities for designing of floral compositions from green spaces, in combination with other spring-flowering species. In addition to plant height, important aesthetic qualities to consider to choosing cultivars include the inflorescence length, colour, and the number of florets in the inflorescence.

The analysis of variance showed significant differences among the nine hyacinth cultivars for the number of days to flowering, duration of flowering, inflorescence length, and the number of florets in the inflorescence. The data related to the evaluation of the main flowering characteristics of the studied cultivars are presented in Table 2.

The number of days from bulb planting to the start of flowering varied from one cultivar to another. The minimum days required for the opening of basal florets (146.16 days) were recorded for 'Purple Pride', which exhibited the earliest flowering, followed by 'Pink Pearl' and 'White Pearl' with an average number of 148.28 and 148.76 days, respectively. The highest number of days to flowering was noticed in the 'Fondant' cultivar, that flowered the latest, after 162.35 days. For the inflorescence length, the range of variance was between 9.53 and 14.33 cm. It was observed that 'Woodstock' was significantly superior to the other cultivars, except 'Pink Pearl' which had a similar value. The lowest inflorescence length was recorded in the 'Fondant' cultivar, and a similar value was reported by Çiğ & Koçak (2019).

The number of florets per inflorescence was recorded when all florets were fully opened and depends on cultivar, but is also influenced by growing conditions and environmental factors especially temperature and light (Luria et al., 2002; Kadam et al., 2013). 'Blue Jacket' and 'City of Haarlem' were the most floriferous cultivars that produced over 40 florets in the inflorescence, while the lowest number of florets (20.16) was observed in 'White Pearl'.

The results indicated that the duration of flowering in the field was different among the nine hyacinth cultivars. 'Fondant' had the longest flowering duration (15.12 days), while the shortest flowering time was recorded at 'Purple Pride' (10.14 days). For the other cultivars evaluated in this experiment, the average flowering duration ranged between 10.38 and 14.33 days.

From the data analysis, it was observed that the lowest coefficient of variation was recorded for the number of days to flowering (3.11%), while the highest coefficient of variation was noticed for the number of florets in the inflorescence (29.68%). The other growth and flowering parameters analyzed exhibited moderate variability. The hyacinth cultivars presented variable responses for the evaluated growth and flowering characteristics, that might be due to the genetic potential and the influence of the environmental conditions, which have an important role in the performance of a cultivar.

Table 2. Evaluation of flowering parameters of various hyacinth cultivars studied under open field conditions

Cultivar	Number of days to flowering	Inflorescence length (cm)	Number of florets per inflorescence	Duration of flowering (days)
Blue Jacket	154.21 b	10.47 bc	44.36 a	12.34 bc
City of Haarlem	150.83 bc	12.26 b	42.50 a	10.86 d
Delft Blue	149.52 cd	11.38 bc	28.65 bc	12.41 bc
Fondant	162.35 a	9.53 c	20.74 c	15.12 a
Jan Bos	152.63 bc	10.65 bc	35.91 ab	10.38 d
Pink Pearl	148.28 cd	14.16 a	32.48 ab	12.73 b
Purple Pride	146.16 d	9.92 c	23.52 c	10.14 d
White Pearl	148.76 cd	11.63 b	20.16 c	14.33 a
Woodstock	152.14 bc	14.33 a	25.23 c	11.25 cd
Mean	151.65	11.59	30.39	12.17
CV%	3.11	14.84	29.68	14.13

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan's multiple range test

CONCLUSIONS

The results of this study revealed significant variations among the nine hyacinth cultivars in terms of the growth and flowering parameters evaluated under similar growing conditions. 'Woodstock' was superior to the other cultivars regarding plant height, as well as the length of leaves and inflorescence. 'Blue Jacket' and 'City of Haarlem' exhibited the highest number of florets per inflorescence.

The minimum number of days to flowering was recorded at 'Purple Pride', while the 'Fondant' and 'White Pearl' cultivars showed the longest flowering duration.

It was concluded that all the studied hyacinth cultivars are suitable for cultivation as spring ornamental plants in green spaces, in containers, and window boxes to provide colour, beauty, and fragrance, but they can be used also as cut flowers and potted plants for the decorating indoor spaces.

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THE BEHAVIOR OF SOME DECORATIVE VARIETIES OF *IPOMOEA BATATAS* IN DIFFERENT CULTURE SYSTEMS AND TYPES OF SUBSTRATE

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Abstract

The study was carried out on three ornamental varieties of Ipomoea batatas (`Heart Bronze`, `Black`, `Heart Lime`) grown in the field and in pots. Four types of substrate were used for pot culture: garden soil + peat, garden soil + peat + hydrogel, garden soil + peat + coconut fiber, garden soil + peat + coconut fiber + hydrogel. In field conditions, the plants from the three varieties were distinguished by a larger size than in the pots. Also, in the field, the higher degree of stem branching was recorded in `Black` and the longest branches in `Heart Lime`. The substrate garden soil + peat favored the length of the branches in all the varieties studied. The garden soil + peat + hydrogel at `Heart Lime` and the garden soil + peat + coconut fiber at `Heart Bronze` had a positive effect on the degree of branching. The use of two-factor ANOVA analysis indicates a strong influence of both the variety and the cultivation system on the morphological characters analyzed.

Key words*: morphological characters, ornamental varieties, sweet potato.*

INTRODUCTION

The Sweet Potato (*Ipomoea batatas* (L.) Lam) belongs to the *Convolvulaceae* family, a large family with approximately 60 genera and over 1650 species (Escobar-Puentes et al., 2022). The genus *Ipomoea* comprises 600-800 species, with *I. batatas* taxonomically placed in the Batatas section, along with 13 other related wild species (Winslow, 2012; Jiang et al., 2022; Firon et al., 2009), namely *I. cordatotriloba*, *I. cynanchifolia*, *I. grandiflora*, *I. lacunosa*, *I. x leucantha*, *I. littoralis*, *I. ramosissima, I. umbraticola*, *I. tabascana*, *I. tenuissima*, *I. tiliacea*, *I. trifida*, *I. triloba* (Khoury et al., 2015; Nimmakayala et al., 2011). Currently, the origin of the sweet potato is widely accepted to be in Central and South America, specifically in the region between the Yucatan Peninsula, Mexico, and the Orinoco River in Venezuela (Austin, 1988, cited by Loebenstein, 2009). Determinations made using molecular markers support the hypothesis that Central America is the main center of origin of this species (Loebenstein, 2009). Some authors do not exclude the existence of secondary diversity centers, which could correspond to areas in China, Southeast Asia, New Guinea, and East Africa (Aguoru et al., 2015).

It appears that it was brought to Spain, and hence Europe, by Columbus, and spread to Africa, India, Southeast Asia, and the Philippines with the help of Portuguese explorers (Escobar-Puentes et al., 2022).

It is primarily cultivated in the tropical regions of Latin America, Africa, and Asia as a food crop (Xiong and Kaluwasha, 2022). Archaeological discoveries in Mexico and Peru indicate the cultivation of the sweet potato from 2500-2000 BC (Nimmakayala et al., 2011; Xiong and Kaluwasha, 2022; Aguoru et al., 2015).

I. batatas is considered a multi-purpose plant, having food, medicinal, and industrial importance (Behera et al., 2022). It occupies an important place in the category of high nutritional value food crops, due to its rich content of starch, proteins, β-carotene, vitamins (B_1, B_2, B_6, C, E), folic acid, essential minerals (Ca, Fe, Mg, Mn, Na, Cu, Zn) etc. (Aguoru et al., 2015; Baley, 2018; Sousa et al., 2019; Nimmakayala et al., 2011; Todesco et al., 2023; Andrade et al., 2017; Vînătoru, 2019). The tuberous roots are prepared in various ways (boiled, fried, baked) or can be turn into flour for the manufacture of bread and other bakery products. They also represent raw material in obtaining food colorants, industrial starch, liquid glucose, citric acid, monosodium glutamate, and alcohol. The leaves are used in animal feed (Behera et al., 2022; Dlamini et al., 2021; Escobar-Puentes et al., 2022; Rosero et al., 2019). The sweet potato also possesses numerous pharmaceutical properties, being used in the treatment of diseases such as diabetes, infections, anemia, hypertension, cancer, aging, allergies etc. (Behera et al., 2022; Escobar-Puentes et al., 2022; Jiang et al., 2022; Todesco et al., 2023).

The sweet potato prefers sunny locations, but also tolerates semi-shade, well-drained, sandy or sandy-loamy soils, and a pH of 5.5-6.5. It requires temperatures of 21-26°C and tolerates drought conditions relatively well, but struggles with excess moisture (Behera et al., 2022; Nedunchezhiyan et al., 2012). However, the sweet potato exhibits a very good adaptability to different environmental conditions, which has allowed its spread to temperate regions and altitudes that can reach 2000-3000 m (Behera et al., 2022; Nimmakayala et al., 2011), or in arid areas, on soils with low fertility, being considered one of the most important crops in ensuring food security in vulnerable areas (Todesco et al., 2023; Rosero, 2019; Glato et al., 2017). Recent studies also establish technologies in an unconventional system, in soilless cultures (Stoian et al., 2022).

I. batatas is a perennial plant, however, it is cultivated as an annual, especially in the temperate-continental climate (Behera et al., 2022; Nimmakayala et al., 2011). It presents tuberous roots with different shapes and sizes, depending on the variety, cultivation conditions, technology etc. The stems are long (1-7 m), cylindrical, usually twisted, green or purple, highly branched and easily form adventitious roots from the nodes upon contact with the soil. The leaves, arranged alternately, have petioles with dimensions of 2.5-20 cm, and the large blade, glabrous or slightly pubescent, oval, circular, triangular, cordate or hastate, entire or palmate-sectate (with 3-7 ovate to linear-lanceolate lobes) (Behera et al., 2022; Jiang et al., 2022; Vîlceanu, 1982; Vînătoru, 2019, Huaman, 1991). Some varieties may present variations in leaf shape within the same plant. The flowers are axillary, funnel-shaped, small in size, white or lavender-violet, with a darker center. The fruit is an ovoid or globular capsule, which opens in 2-4 valves (Buia et al., 1965; Behera et al., 2022).

The species is self-incompatible, and seeds are formed only when compatible varieties are cultivated for crossbreeding (Martin, 1965). Therefore, varieties of *I. batatas* are usually propagated vegetatively, by cuttings made from stems or from tuberous roots (Jiang et al., 2022; Behera et al., 2022).

Being a hexaploid species (2n = 6x = 90), *I. batatas* has a great variability of characters, especially in terms of the size, color and shape of leaves and stems, the size, shape, color and production of tuberous roots etc. (Rosero et al., 2019). Except green, leaves and stems have colors that vary from white to yellow, orange or brown-orange and from pink to red-violet or intense violet (Jiang et al., 2022). Therefore, through artificial or natural selections, a large number of varieties have been obtained, which differ both in morphology and in the chemical composition of the tuberous roots (Jiang et al., 2022).

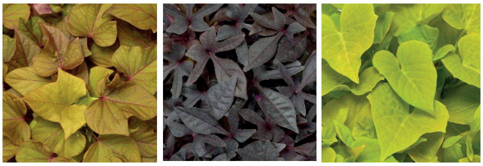
This explains the increasing interest in the ornamental use of some varieties of *I. batatas*, characterized by their beautiful foliage, persistent throughout the growing season and by the interesting aspect of the bushes, with a semi-erect or compact habit, trailing or climbing, with stems that can reach lengths of 3 m or more. Although they are usually used for their decorative foliage, some varieties are also capable of flowering (Huaman, 1991).

The sweet potato has a great ornamental potential (Sousa et al., 2018) if it is properly valued and offers a wide range of possibilities in the arrangement of gardens, terraces, and balconies. Its rapid growth is a major advantage in ensuring decor in a relatively short time, in hanging pots, flower pots, containers, and planters, or as a ground cover plant. It is a suitable choice for color spots, and the varieties with long stems for covering walls, pergolas, arches, etc. Recently, it is also found in the assortment of plants for arranging green walls (Cojocariu et al., 2024).

Despite all the advantages it offers, the culture of sweet potato is less widespread in Romania, for a long time being almost unknown as an ornamental plant, and as a food plant treated in the category of less widespread vegetables (Vîlceanu, 1982). However, in recent years, interest in this plant has increased, either for the production of tuberous roots (Vînătoru, 2019; Stoian et al., 2022), or for decorative purposes (Cojocariu et al., 2024; Ozarchevici et al., 2022). This paper aims to highlight a series of morpho-decorative characters of some ornamental varieties of *I. batatas*, under the conditions of their cultivation in the field and in pots, on different types of substrate.

MATERIALS AND METHODS

The plant material was represented by three varieties of *I. batatas*: 'Heart Bronze', 'Black', and 'Heart Lime'.



a) b) c)

Figure 1. a) 'Heart Bronze', b) 'Black', c) 'Heart Lime' (https://www.syngentaflowers.com/products/search/flower?keywords=sidekick&items_per_page=12)

'Heart Bronze' (Figure 1a) is a variety with a very high branching power, appreciated for its uniform habit and dense foliage, with a fine texture, in unique shades. At maturity, it can reach approximately 30 cm in height, with a branch length of 60-90 cm, even more under optimal conditions. The leaves are hastate, moderately lobed, with khaki green and ruby red or burgundy shades.
'Black' (Figure 1b) is characterized by its semi-erect habit, special foliage, and the ability to bloom throughout the growing season. The leaves, violet in color, are deeply lobed, thus giving the plant a "lacy" appearance. At maturity, the bush reaches up to approximately 30 cm in height, and the branches to lengths of 50-60 cm.
'Heart Lime' (Figure 1c) is a variety with long branches and a large covering capacity. At maturity, the plant can reach approximately 30 cm in height, with a branch length of 75-120 cm. The leaves are cordate or hastate, slightly lobed, lime green in color and ruby-red shades on the edge of the limb.

The composition of the substrates for pot culture was based on the combination of the following components: peat, coconut fiber, garden soil, and hydrogel.
SuliFlor peat (SF2) was used, with pH = 5.5-6.5 and medium structure (0-20 mm), improved with complex fertilizers (1.5 kg/m^3 NPK 14-16-18) and additives based on limestone and dolomite powder.
Dehydrated coconut fiber (Neopeat), supplied by Kertimag, was characterized by pH = 5.5-6.5 and water retention capacity of 650-850%.
The granulated hydrogel, an ecological water absorbent polymer based on potassium, supplied by Gardenis, presented a neutral pH and the density of the filtering surface of 30-60 mesh.
The garden soil was collected from the same area where the field crops were established.
The experimental cultures were established in 2023, in the experimental field of the Floriculture discipline, within the Iași University of Life Sciences, Romania (47°11′31″ N, 27°33′20″ E latitude, in temperate-continental climate with excessive nuances). For the establishment of the cultures, rooted cuttings were used, purchased from specialized companies (Syngenta Company).
In field conditions, the planting of the cuttings was done on ridges, at 80 cm between rows and 40 cm between plants in a row.
The pot culture was established in pots with a volume of 5 L and in different types of substrate.
The field experience was monofactorial, the experimental factor being represented by the variety, with three graduations, resulting in three experimental variants noted with the initials of the name: HB ('Heart Bronze'), B ('Black'), HL ('Heart Lime').
In the case of potted plants, the experience was bifactorial, one of the factors being the variety (with the three graduations and with the symbols presented earlier), and the other, the type of substrate resulting from the combination of the components symbolized by the initials of the name: GS (garden soil), P (peat), C (coconut fiber), H (hydrogel). The combinations made, respectively the variants, were the following: GSP (garden soil + peat), GSPH (garden soil + peat + hydrogel), GSPC (garden soil + peat + coconut fiber) and GSPCH (garden soil + peat + coconut fiber + hydrogel). In each variant of substrate, the components represented equal

parts in terms of volume, with the exception of the hydrogel, where the amount added to the mixture was 2 g/L.
The soil from the experimental field, namely the garden soil (GS) used in the mixtures for potted plants, is a cambic chernozem, with sandy-loamy texture and slightly alkaline pH (7.8). The mixtures that constituted the substrates of the potted cultures had a neutral to slightly alkaline pH (7.2-7.8). Regarding the content of main macronutrients, according to ICPA Bucharest (National Institute of Research - Development for Pedology, Agrochemistry and Environmental Protection), the level of total N (%), respectively P and K (mg/kg) falls within the good and very good level.
The experiments were organized in randomized blocks with three repetitions, with 9 plants/repetition.
Observations and determinations were made from the moment of setting up the experiments (May) until the end of the vegetation season (October). These were on biometric indices represented by the number of branches per plant and the length of the stems.
To establish the relationships between the analyzed characters, the scatter diagram of the values and mathematical modeling through linear regression and testing of the variant (ANOVA one-way) were used.
The association between a dependent variable and two independent variables was analyzed, using the ANOVA Two-Factor with Replication test which determines the influence of the first factor, the influence of the second factor, as well as the combined influence of the two factors. The statistical testing was performed with a significance level of 0.05, using the MS EXCEL professional application from the MS OFFICE 2019 package.

RESULTS AND DISCUSSIONS

The observations and determinations made aimed to evaluate the decorative effect of the three varieties of *I. batatas* cultivated either in the field or in pots, in different substrate compositions.
In Table 1, the absolute average values of the maximum length of the stems and the number of branches/plant are presented, recorded following the determinations made for all experimental variants from the field and pots. For all varieties, the plants grown in pots in GSP substrate formed the longest stems. The least favorable influence for this character was the GSPCH substrate. Cultivated in the field, the HB and B varieties recorded stem lengths with intermediate values between the maximum and minimum of those from pots, respectively 70.5 cm at HB and 52.5 cm at B. In contrast, the HL variety stands out with a stem length 2-3 times larger than the variants from pots (187 cm). This determined that the average length of the stems at the potted plants (65.7 cm) to be approx. 22% below the value of those in the field (84.5 cm).

Table 1. Stem length and number of branches (absolute values)

Cultivars	Growing system	Substrates	Stems length (cm)	Number of branches/plant (pc.)
HB	Pots	GSP	78.0	9.7
		GSPH	58.0	9.0
		GSPC	58.7	11.3
		GSPCH	51.7	10.3
	Field	-	70.5	6.0
B	Pots	GSP	72.7	5.0
		GSPH	51.7	6.0
		GSPC	61.3	5.3
		GSPCH	50.0	5.7
	Field	-	52.5	7.0
HL	Pots	GSP	86.7	8.3
		GSPH	80.3	10.0
		GSPC	76.0	8.3
		GSPCH	62.7	7.1
	Field	-	187.0	6.0
Average	*Pots*	-	*65.7*	*8.1*
	Field	-	*84.5*	*6.3*

The number of branches/plant had larger variations, depending on the variety, cultivation system, and substrate (Table 1). The GSPH substrate influenced the degree of branching of the stems at the potted plants of the B and HL varieties, while at HB the maximum values (11.3) were obtained in the GSPC substrate. Under field conditions, the degree of branching was below the value of the plants grown in pots for the HB and HL varieties, but larger at B. The average number of branches at the potted plants was larger than at the plants grown in the field (8.1, respectively 6.3).
In Tables 2-4, the relation between the stem length and the type of substrate used for potted cultures was analyzed for each variety. The analysis of variance (ANOVA) led to values smaller than the significance level of 0.05 and thus the hypothesis of equality of means was rejected. For this reason, the post-hoc Tukey

analysis was applied, which highlighted the data groups that differ as an average. The values for p-*values* obtained following the tests were centralized in Tables 2-8.

In the case of the HB variety, it can be observed that there are significant differences between all the resulting pairs, with the exception of the pair formed from GSPH and GSPC, where there is a similarity of 0.93.

Table 2. Relationship between the length of the stems and the type of substrate (`Heart Bronze`)

	HB GSPH	HB GSPC	HB GSPCH
HB GSP	0.00000*	0.00000*	0.00000*
HB GSPH		0.93070	0.00007*
HB GSPC			0.00001*

*Level of significance 0.05.

For the B variety (Table 3), as in the previous case, significant differences are observed between all pairs of substrate, this time with the exception of the pair formed from GSPH and GSPCH, with a value of 0.34.

Table 3. Relationship between the length of the stems and the type of substrate at (`Black`)

	B GSPH	B GSPC	B GSPCH
B GSP	0.00000*	0.00000*	0.00000*
B GSPH		0.00000*	0.34027
B GSPC			0.00000*

*Level of significance 0.05.

The HL variety behaves similarly to the HB variety, with significant differences for all pairs of substrate, with the exception of the pair formed from GSPH and GSPC, with a similarity of 0.66 (Table 4).

Table 4. Relationship between the length of the stems and the type of substrate (`Heart Lime`)

	HL GSPH	HL GSPC	HL GSPCH
HL GSP	0.00333*	0.00000*	0.00000*
HL GSPH		0.066340	0.00000*
HL GSPC			0.00000*

*Level of significance 0.05.

In a similar manner, the number of branches per plant was analyzed for each type of substrate, for each variety (Tables 5-7). For HB (Table 5), a similarity of approximately 0.21 was recorded for the substrate pairs formed from GSP with GSPH, respectively with GSPCH. The pair formed from GSPC and GSPCH recorded a similarity of approximately 0.67.

Table 5. Relationship between the number of the branches and the type of substrate (`Heart Bronze`)

	HB GSPH	HB GSPC	HB GSPCH
HB GSP	0.20917	0.01794*	0.20917
HB GSPH		0.00007*	0.00189*
HB GSPC			0.66632

*Level of significance 0.05.

In the case of the B variety (Table 6) however, the differences are significant only between the pair formed by GSPH and GSPCH.

Table 6. Relationship between the number of the branches and the type of substrate (`Black`)

	B GSPH	B GSPC	B GSPCH
B GSP	0.5501	0.35735	0.55000
B GSPH		0.75052	0.00189*
B GSPC			0.66632

*Level of significance 0.05.

The HL variety showed a behaviour similar to the HB variety, with the exception of the pair formed by GSPC and GSPCH, which had a similarity of approximately 0.75.

Table 7. Relationship between the number of the branches and the type of substrate (`Heart Lime`)

	HL GSPH	HL GSPC	HL GSPCH
HL GSP	0.20917	0.01794*	0.20917
HL GSPH		0.00007*	0.00000*
HL GSPC			0.75052

*Level of significance 0.05.

Through the Anova Single Factor analysis, the interaction between stem length and the number of branches per plant was also evaluated, depending on the variety and cultivation system (Table 8 and Table 9).

Table 8. Interaction between the length and number of branches according to the cultivar (in the pots)

	Stem length		No. of branches	
	B	HL	B	HL
HB	0.65086	0.17134	0.00000*	0.00206*
B		0.02129*		0.00000*

*Level of significance 0.05.

Table 9. Interaction between the length and number of branches according to the cultivar (in the field)

	Length of stems		Nr. of branches	
	B	HL	B	HL
HB	0.986992	0.81067	0.61624	0.00000*
B		0.72090		0.00000*

*Level of significance 0.05.

For plants grown in pots (Table 8), the length of the branches recorded significant differences only between the B and HL varieties, while the number of branches showed significant differences for all pairs of varieties formed (p-*value* < 0.05).

In the field crops (Table 9), the length of the branches between varieties shows a similar evolution for all pairs formed, reaching values of approximately 0.99 between the HB and B varieties. For the number of branches, were observed significant differences for all pairs of varieties, with the exception of the first pair formed from the HB and B varieties.

In order to reveal the effect of both substrate type and variety on the number of branches per plant, an ANOVA Two-Factor with Replication analysis was performed. Regarding the influence of the substrate type and the variety on the number of branches in potted plants (Figure 2), the p-*value* indicates significant differences (p-*value* < 0.05), as both the column interaction, which represents the substrate types, and the interaction between varieties influence the number of branches per plant.

SUMMARY	GSP	GSPH	GSPC	GSPCH	Total	
HB						
Average	9,66667	9	11	10,5556	10,055556	
Variance	0,25	0,8	1	0,52778	1,1968254	
B						
Average	5	5,9	5,55556	5,88889	5,5833333	
Variance	0	0,6	0,27778	1,11111	0,5928571	
HL						
Average	8,44444	10	8,44444	7,66667	8,6388889	
Variance	0,27778	0,8	0,27778	0,25	1,0944444	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	376,13	2	188,065	370,977	6,83E-46	3,091
Columns	6,85185	3	2,28395	4,50533	0,0053071	2,699
Interaction	45,4259	6	7,57099	14,9346	5,384E-12	2,195
Within	48,6667	96	0,50694			
Total	477,074	107				

Figure 2. Anova Two- Factor for the number of the branches according to the cultivar and the type of substrate (in the pots)

The analysis of stem length in potted plants indicates significant differences (0.0053) in the interaction between variety and substrate (Figure 3).

Also, using ANOVA Two-Factor with Replication analysis, it was determined the effect of the cultivation system and variety on the number of branches per plant and the length of the stems. For both characters analyzed, the p-*value* values indicate significant differences (Figure 4 and Figure 5), which indicates a strong influence of both the variety and the cultivation system on the analyzed morphological characters.

SUMMARY	GSP	GSPH	GSPC	GSPCH	Total	
HB						
Average	78	58	58,73333	51,7333	61,616667	
Variance	3,2025	10	10,1975	3,31	105,74543	
B						
Average	72,7	51,7	61,33333	50	58,933333	
Variance	4,34	5,76	4,6925	3,2075	88,304	
HL						
Average	86,7	80,3	76	62,7	76,433333	
Variance	15,56	18,5	10,625	6,0275	91,153714	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6395,81	2	3197,903	402,147	2,181E-47	3,091
Columns	8226,46	3	2742,152	344,834	2,948E-51	2,699
Interaction	992,253	6	165,3756	20,7965	1,759E-15	2,195
Within	763,4	96	7,952083			
Total	16377,9	107				

Figure 3. Anova Two-Factor for the length of the stems according to the cultivar and the type of substrate (in the pots)

SUMMARY	Field	Pots	Total			
HB						
Average	6	10,083	8,04			
Variance	0,1073	1,3561	5,05			
B						
Average	7	5,5	6,25			
Variance	0,0891	0,6364	0,93			
HL						
Average	6,05	8,5833	7,32			
Variance	0,0536	1,1742	2,26			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	38,988	2	19,5	34,233	6,3E-11	2,748
Columns	52,361	1	52,4	91,95	4E-14	3,991
Interaction	99,688	2	49,8	87,531	2,7E-19	2,748
Within	37,583	66	0,57			
Total	228,62	71				

Figure 4. Anova Two- Factor for the number of the branches according to the cultivar and the growing system

SUMMARY	Field	Pots	Total			
HB						
Average	70,5	61,617	66,05833			
Variance	5,3291	106,85	74,23645			
B						
Average	52,5	58,933	55,71667			
Variance	2,8164	90,795	55,56754			
HL						
Average	188,08	76,433	132,2583			
Variance	31,174	86,604	3308,256			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	82784	2	41392,07	767,543	2E-46	3,136
Columns	26038	1	26037,62	482,822	4,6E-32	3,986
Interaction	49479	2	24739,26	458,746	1,9E-39	3,136
Within	3559,2	66	53,92798			
Total	161860	71				

Figure 5. Anova Two-Factor for the length of stem according to the cultivar and the growing system

For the two characters analysed, similar studies in the literature on edible sweet potato varieties indicate approximately close values.

Regarding the number of branches, Shitikova (2022) reported 4 - 10 branches per plant in six sweet potato varieties, the number varying according to the variety used.

Gobena (2022), in a study on the adaptability of edible sweet potato varieties, reports branch length values between 56.3 and 143 cm. Similar values for edible varieties were also obtained by Shamil (2021) with length ranges between 135.2-175.1 cm and Nazrul (2018) with lengths of 119-192.3 cm, arguing that these differences may be due to both the genetic make-up of the genotypes and climatic conditions.

Another aspect analyzed was the tendency of variation in morphological characters (stem length and number of branches) depending on the type of substrate used in potted cultures (Figure 6).

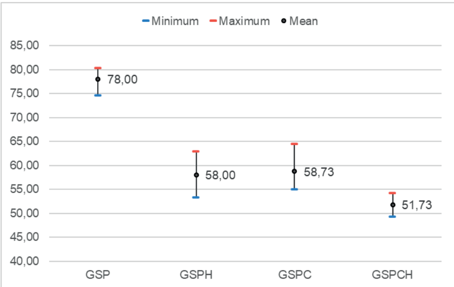


Figure 6. The minimum and maximum values for the lenght of stems according to the type of substrate (`Heart Bronze`)

For the HB variety, there is a tendency towards maximum values of stem length (78 cm) in the case of the GSP substrate, and a tendency towards minimum values (51 cm) in the case of the GSPCH mixture (Figure 6).

In the case of the number of branches per plant for the HB variety, the highest values (11) were recorded for the GSPC substrate. The minimum values (9) were for plants grown in the GSPH substrate (Figure 7).

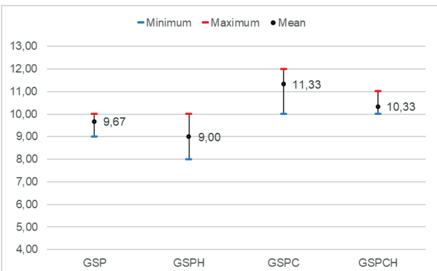


Figure 7. The minimum and maximum values for the number of branches/plant according to the type of substrate (`Heart Bronze`)

The trend towards maximum and minimum values of stem lengths for the B variety (Figure 8) is similar to that of HB, in the sense that the maximum values (72.7 cm) are in the GSP substrate, and the minimum ones (50 cm) are in the GSPCH substrate.

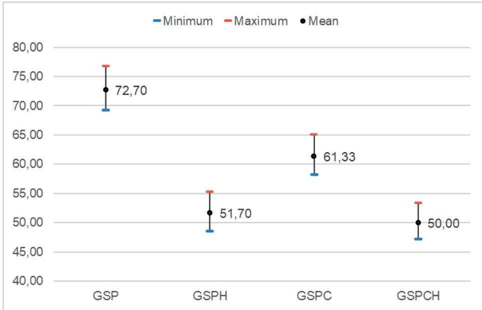


Figure 8. The minimum and maximum values for the lenght of stems according to the type of substrate (`Black`)

From the analysis of the number of branches in the B cultivar (Figure 9), a different reaction to the type of substrate was observed compared to the previous cultivar. Despite the fact that the disparities among the variants were not substantial, being between 5 and 6, the maxi-//mum values were determined by the GSPH substrate, and the minimum ones by the GSPC substrate.

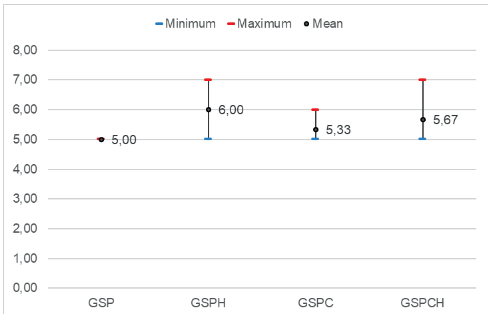


Figure 9. The minimum and maximum values for the number of branches/plant according to the type of substrate (`Black`)

With stem lengths ranging from 62.7-86.7 cm, the trend towards maximum values in HL was ensured by the GSP substrate, and the minimum values by the GSPCH substrate, somewhat similar to the other two cultivars, only with differences in the intermediate values from GSPH and GSPC (Figure 10).

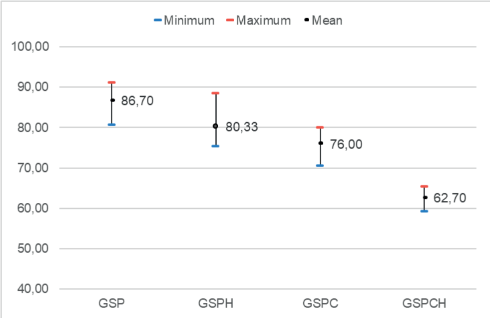


Figure 10. The minimum and maximum values for the lenght of stems according to the type of substrate (`Heart Lime`)

The degree of stem branching in the HL cultivar was higher in plants grown on the GSPH substrate, with an average of 10 branches per plant. On the GSPCH substrate, the values were minimal, with an average of 7.67 branches per plant (Figure 11).

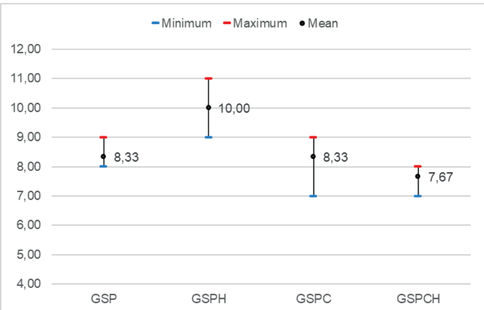


Figure 11. The minimum and maximum values for the number of branches/plant according to the type of substrate (`Heart Lime`)

The study also included the analysis of existing correlations between the morpho-decorative traits of *I. batatas* varieties and corresponding linear regressions were established. Pearson correlation coefficients were calculated and regression equations were written. In Figure 12, it is observed that between the stem length and the number of branches in the HB variety, the correlation is weak, but inverse (r = –0.22), the coefficient of determination being R^2 = 0.0516, such that the increase in stem length is weak correlated with the decrease in the number of branches.

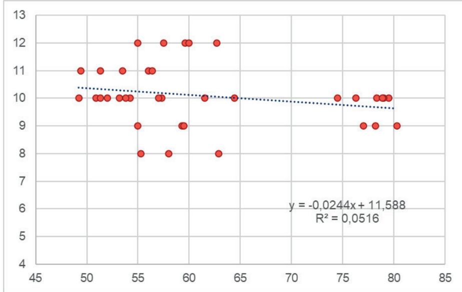


Figure 12. Correlation between length and number of the branches per plant (`Heart Bronze`)

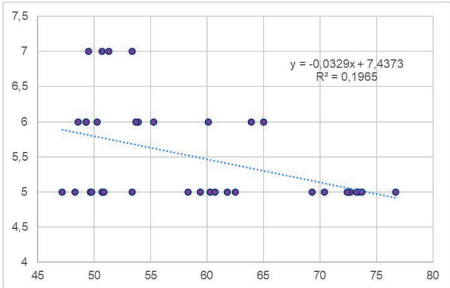


Figure 13. Correlation between length and number of the branches per plant (`Black`)

An average indirect correlation was observed between the analyzed traits in the B variety (Figure 13), with a correlation coefficient of r = –0.44. The determination coefficient is R^2 = 0.1965. This suggests a moderate negative relationship between the traits in this variety. The determination coefficient indicates that approximately 19.65% of the variation in one trait can be explained by the variation in the other trait.

The HL variety was the only one where the increase in stem length was associated with the increase in the degree of branching (Figure 14), the correlation coefficient being r = 0.36, show an average direct correlation and the determination coefficient being R^2 = 0.1262. This suggests a moderate positive relationship between stem length and degree of branching in this particular variety. The determination coefficient indicates that approximately 12.62% of the variation in the degree of branching can be explained by the variation in stem length.

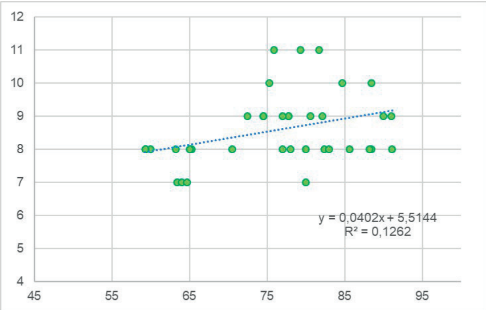


Figure 14. Correlation between length and number of the branches per plant (`Heart Lime`)

CONCLUSIONS

Regardless of the variety, the plants grown in pots in a substrate composed of garden soil and peat (GSP) formed the longest stems.

In field conditions, the 'Heart Lime' (HL) variety stands out through the stem length being 2-3 times larger than the pot variants (187 cm).

The substrate composed of garden soil, peat, and hydrogel (GSPH) favored the degree of stem branching in the plants grown in pots from 'Black' and 'Heart Lime', while in the 'Heart Bronze' variety the maximum values (11.3) were obtained in the GSPC substrate (garden soil, peat, and coconut fiber).

According to the results from the two analyzed characters, the presence of the hydrogel is only justified in combinations with garden soil and peat (GSPH) for the HL variety, and in combination with garden soil, peat, and coconut fiber (GSPCH) for the B variety, but only for better stem branching. It is not recommended for the HB variety.

Among the four substrates used for pot cultivation, the weakest results were recorded, in most cases, for the GSPCH variant.

Grown in the field, the 'Heart Bronze' and 'Heart Lime' (HL) varieties formed a smaller number of branches than those cultivated in pots, while 'Black' recorded higher values.

The correlation coefficients between stem length and the number of branches per plant indicate the presence of a direct correlation in 'Heart Lime' and an indirect correlation in 'Heart Bronze' and 'Black'.

The appropiate use for ornamental purposes of some *I. batatas* varieties studied will take into account both the genotypic characteristics (longer stems in HL, higher degree of branching in HB) and the culture system (longer stems in field for HL and for HB and B in pots; higher number of branches for B in the field and for HB and HL in pots).

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COLOUR PREFERENCE AND RESPONSE OF THRIPS TO DIFFERENT PETUNIA VARIETIES IN WESTERN ROMANIA

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Abstract

Thrips are a common pest in most horticultural and ornamental crops. They are particularly common in greenhouses in western Romania. In the present study, we investigated thrips preference for different coloured traps as well as specific structure and abundance in Petunia hybrida varieties in greenhouses. Thrips attraction to colour was assessed using three commercially available coloured sticky traps: yellow, orange and blue. Taxonomically, the diversity of thrips is reflected in the relatively large number of species, identified in Petunia, 6 species: Frankliniella intonsa, Frankliniella occidentalis, Frankliniella schultzei, Scirtothrips dorsalis, Thrips palmi and Thrips tabaci. Of these species, 2 are the most common and abundant: Frankliniella occidentalis (n= 172) and Thrips tabaci (n= 163). In the Petunia varieties, significant differences were observed depending on the colour of the trap, these were found between yellow-blue (p = 0.000) and yellow-orange (p = 0.000) and it is observed that there are no significant differences between blue-orange (p = 0.434). Most of the thrips species were caught with the yellow sticky traps (F=39.398, p=0.000<0.05).

Key words:*colour preference, Petunia hybrida, thrips, western Romania.*

INTRODUCTION

The current world checklist of thrips contains approximately 7700 species (Tang et al., 2023), grouped in 2 suborders and 9 families (Mound, 2007; Diffie et al., 2008). The suborder *Tubulifera* comprises a single family, *Phlaeothripidae*, with 3500 species (Morse & Hoddle, 2006; Tipping, 2008). The suborder *Terebrantia* comprises 8 families: *Thripidae* (≈2201 species), *Aeolothripidae* (≈222 species), *Heterothripidae* (93 species), *Melanthripidaea* (79 species); *Stenurothripidae* (24 species); *Merothripidae* (20 species), *Fauriellidae* (5 species) and *Uzelothripidae* (2 species) (Buckman et al. 2013).

The most common species associated with ornamental plants as hosts are mainly from three of the nine families of the *Thysanoptera*, with the majority of species from the family *Thripidae* (He et al., 2020). Species in this family are among the most highly evolved of the *Thysanopter*a (Ebratt-Ravelo et al., 2019). They are also the most common and difficult phytophagous insects to control in commercial ornamental production, feeding on a wide range of plant species including bedding plants, potted plants and cut flowers (Parker et al., 1995).

Petunias (*Petunia hybrida*) are among the most popular bedding plants in the world due to their versatility, variety and range of flower colours (Kessler, 1999). In Romania, petunias have been among the top five selling bedding plants for the last 20 years (Popescu & Popescu, 2015), and in this case the popularity and demand for petunias is increasing, leading to the expansion of this plant's cultivation area. However, it has been reported that the aesthetic and ornamental value of petunias can suffer significant economic losses due to thrips infestation.

Four species: *Frankliniella occidentalis* Pergande, *Thrips tabaci* Lindeman, *Thrips palmi* Karny and *Scirtothrips dorsalis* Hood, all of the family *Thripidae*, are the most economically important species in nurseries because of their widespread distribution, highly polyphagous life cycle, strong ability to transmit viruses and severe damage to crops. (Morse & Hoddle, 2006; Wu et al., 2018, 2021; Vîrteiu et al., 2022).

Damage caused by their feeding can have a negative impact on world trade due to the quarantine risks associated with some of the species (Kumar et al., 2012). In order to apply the most effective control strategy, it is important to know which thrips species are present in the nursery. So, the aim of this study was to determine the preference for different coloured traps of *Thripidae* assemblages present in *Petunia hybrida* varieties in Timiș County, Western Romania nurseries, as well as their structure and abundance.

MATERIALS AND METHODS

Sampling of thrips for species identification was carried out at commercial bedding plant greenhouses near Timișoara, Western Romania (latitude: 45°50'16"N; longitude: 21°06'02"E; altitude: 99 m above sea level). The greenhouse was managed conventionally and ornamental plants were grown on tabletops in small pots covering a total of 98 m^2, of which *Petunia hybrida* plants covered 21 m^2.

Crop management included the use of bio-stimulants and fertilizers such as Algaren Twin (25 ml/10 l water) mixed with MagicP Star (10 ml/10 l water) at transplanting; King Life 12 - 48 - 8 + Mg (10 g/10 l water) and Calfomyth (35 ml/10 l water) at 10-15 days after transplanting; two further treatments with Drin (15 ml/10 l water), Foliacon 22 (25 ml/10 l water) and King Life 30 - 10 - 10 + Mg (7-10 ml/10 l water) at 10-15 days after the first treatment; King Life 20 - 20 - 20 (7-10 g/l water) and Calfomyth (35 ml/10 l water) at bud burst; King Life 8 - 5 - 40 + Mg (10 ml/10 l water); Algaren Twin (15 ml/10 l water) and Calfomyth (35 ml/10 l water) at flowering; while no chemical pest control was applied.

Seasonal and temporal sampling of thrips adults and larvae was used. Thrips samples were taken from the leaves and flowers of 12 varieties of *Petunia hybrida*, namely: 'Tropical', 'Beautical Mix 2', 'Calipetite Mix 1', 'Calipetite Mix 2', 'Contrast Mix 1', 'Purple Picotee', 'Double Red', 'Cream Picotee', 'Cherry Pop', 'Bicolor Yellow Red', 'Orange Bouquet' and 'Pink Bouquet'. Three double-sided sticky traps of different colours: blue, yellow and orange (10.2 × 20.5 cm in size) were placed at a distance of 0.5 m from each other in the middle of each plot/petunia variety. Each plot consisted of 30 petunias (Parnea et al., 2018; Vîrteiu et al., 2018; Muntean & Grozea, 2021; Vîrteiu et al., 2021). As the thrips species spread differently in the nursery, 3 traps (one trap/colour) per petunia variety were evenly distributed around the perimeter of a plot (Figure 1).

Figure 1. Sampling by the sticky trap method

The traps were attached to a cable so that their lower edge was 10-15 cm below the tops of the plants (Vîrteiu et al., 2022). Traps were monitored and replaced every 10-15 days (all over sampling period) and repeated seven times between February and May 2023 (sampling dates: 20 February, 6 March, 22 March, 8 April, 24 April, 8 May and 22 May). After removing the coloured sticky traps, they were wrapped in clear plastic cling film and transported to the laboratory for thrips count (Pobozniak et al., 2020).

All specimens of thrips were identified to the species level according to the standard identification keys provided by Mound and Marullo (1996), Cluever & Smith (2017). Once the data had been processed, the insects were preserved by preparing slides as follows: 50 specimens were randomly selected from each trap, 25 from each side of the trap (a solvent was used to remove the specimens - HistoClear II - so as not to damage them), and then mounted on slides using Canada balsam as a mounting medium. If less than 50 thrips were present per trap, as in the first part of the collection, all were removed and identified.

RESULTS AND DISCUSSIONS

Thrips populations collected from petunia plants, under controlled conditions, were not very high in comparison to other open ecosystems (agricultural crops, pastures, groves). Virteiu et al. (2015) highlighted the presence of 12 thrips species on rose flowers, and Bărbuceanu & Vasiliu - Oromuru (2002) also collected a number of 12 thrips species on ornamental plants in the southern part of the country. In the climatic conditions of the western part of the country, other authors (Parnea et al., 2018; Virteiu et al., 2018) highlighted between 8 and 15 thrips species on oat and winter wheat. Some experimental parameters, such as: plant density, plot size and plot type had an important role in the development of thrips populations. The research carried out by Alvi et al (2021) showed that increasing row spacing leads to an increase in the density and number of thrips species, while the research carried out by Belder et al (2002) on a series of 43 agricultural landscape plots showed that the number of different thrips species was significantly lower in agricultural fields with a larger total area compared to woodland, which reduces the density of thrips and their symptoms.

Due to the specific microclimate, the taxonomic spectrum of thrips species was relatively rich and diverse. Thus, the collected material revealed a numerical abundance of 498 specimens: 394 of these were adults and 144 were larvae of different ages. From a taxonomic point of view, the diversity of the thrips species was reflected in the relatively high number of species identified on petunias - 6 species: *Frankliniella intonsa* (Hinds), *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Trybom), *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny and *Thrips tabaci* Lindeman.

Of these species, 2 were the most abundant in the sample: *Frankliniella occidentalis* with an average of 5.11 specimens/traps and *Thrips tabaci* with average specimens/traps of 4.86, while *Frankliniella schultzei* presented the fewest specimens (0.083 specimens/traps). The remaining 3 species (*Frankliniella intonsa, Scirtothrips dorsalis* and *Thrips palmi*) were moderately abundant with an average of 2.13, 1.55 and 0.75 specimens/ traps. This particular climate had an impact on the specific structure of the thrips species. *Thrips dorsalis* Hood and *Thrips palmi* Karny, mesophile species, were collected for the first time from petunias in western Romania in early May, when temperatures in the greenhouse increased considerably.

Table 1. Thrips species structure in relation to *Petunia hybrida* plants

Thrips species	N*	Mean*	Std. Deviation	95% Confidence Interval for Mean		Min. *	Max. *	H'*
				Lower Bound	Upper Bound			
Frankliniella occidentalis	36	5.1111	4.01979	3.7510	6.4712	0.00	14.00	2.39
Frankliniella intonsa	36	2.1389	3.22626	1.0473	3.2305	0.00	12.00	0.00
Frankliniella schultzei	36	0.0833	0.36839	-0.0413	0.2080	0.00	2.00	0.00
Thrips tabaci	36	4.8611	4.49859	3.3390	6.3832	0.00	17.00	2.43
Thrips palmi	36	0.7500	1.85742	0.1215	1.3785	0.00	8.00	0.00
Scirtothrips dorsalis	36	1.5556	1.90405	0.9113	2.1998	0.00	6.00	0.00
Total	216	2.4167	3.53882	1.9421	2.8913	0.00	17.00	1.46

ANOVA	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	801.944	5	160.389	17.816	.000
Within Groups	1890.556	210	9.003		
Total	2692.500	215			

* N - number of samples analysed; Mean - mean number of specimen/ sample; Min. - minimum number of specimens/ sample; Max - maximum number of specimen/ sample; H' - Shannon Diversity Index

The data in Table 1 underline the existence of significant differences between number of specimens and thrips species (F=17.816, p=0.000<0.05).

Analysis of thripsofauna composition revealed the constant presence of *Frankliniella occidentalis* and *Thrips tabaci* in all samples, with different population sizes and the highest structural index values. *Thrips tabaci* and *Frankliniella occidentalis* had the highest values (2.43-2.39). This high index highlights the significant differences between the species constituting the thrips fauna. The species *Frankliniella schultzei, Thrips palmi, Scirtothrips dorsalis, Frankliniella intonsa* had the lowest value. This highlights the sporadic occurrence of these species in the samples and also reflects a dynamic fauna, as these polyphagous species do not only attack the petunias, but also the other plant species present in the greenhouse.

The negative binomial distribution of this group of insects was also evidentiated by the numerical values of the comparative indices between the species that make up the associations of thrips on petunia inflorescences.

From an ecological point of view, the fauna of the *Thripidae* on petunia inflorescences was dominated by species that are typically floriphagous, among which *Frankliniella occidentalis, F. intonsa* and *Thrips tabaci* stand out. *Frankliniella schultzei* was present accidentally, probably because the associated plants growing around the greenhouse allowed this species to develop.

Vîrteiu et al. (2022) reported that all 6 thrips species were associated with *Petunia hybrida* varieties in nurseries in the western part of Romania. All the six species were affiliated to: three species with *Frankliniella* genus (*F. occidentalis, F. intonsa, F. schultzei*), two species with the genus *Thrips* (*T. tabaci* and *T. palmi*), one species with the *Scirtothrips* genus and, considering the survey reported by Funderburk et al. (2007), they were collected from almost all ornamental greenhouses.With regard to *Frankliniella occidentalis* being the most abundant species in petunias in greenhouses, Bărbuceanu & Vasiliu-Oromulu (2012) highlighted that this species is now present in most greenhouses in Romania, eliminating another thrips species from these protected areas, as *Heliothrips haemorrhoidalis* (Heeger), *Thrips dianthi* (Priesner), *Parthenothrips dracaenae* (Heeger), *Heliothrips feromoralis* (Renter). *T. tabaci* has been described to be a polyphagous and cosmopolitan species (Vîrteiu et al., 2015), with the second highest occurrence in the petunia studied and in all 12 varieties. *Scirtothrips dorsalis* and *Thrips palmi* are also reported to be present on the flowers of ornamentals (Vasiliu-Oromulu, 2002). With the exception of one species, *Frankliniella intonsa*, which is strongly xerophilic, the fauna of this group of insects is dominated by mesophilic species in terms of climatic preferences. The increased temperatures in early May did not affect the mesophilic species that continued to occur in the samples, although some of them occurred sporadically.

Table 2. Analysis of multiple comparisons between different species of thrips

	Thrips species	Number of samples analysed	Subset	
			1	2
Tukey HSD[a,b]	*Frankliniella schultzei*	36	0.0833	
	Thrips palmi	36	0.7500	
	Scirtothrips dorsalis	36	1.5556	
	Frankliniella intonsa	36	2.1389	
	Thrips tabaci	36		4.8611
	Frankliniella occidentalis	36		5.1111
	Sig.		0.069	0.999

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 9.935.
a. Uses Harmonic Mean Sample Size = 36.000.
b. Alpha = 0.05.

A statistically significant relationship between the thrips species was found using the Tukey HSD test (Table 2). Therefore, any numerical change in thrips species composition was causally related to a change in one of the individual species values.

When analysing the abundance of thrips species in the 12 varieties of petunia included in the study, it was found that the variety with the highest abundance of thrips species was 'Calipetite Mix 2' with an average of 3.77 and a standard deviation of 4.023, followed by 'Double Red' with an average of 3.38 and a standard deviation of 4.81 (Table 3). The varieties with the least thrips infestation were

'Pink Bouquet' with an average of 1.33 individuals and a standard deviation of 2.72 and 'Cream Picotee' with an average of 0.88 individuals and a deviation of 2.16. The analysis of the variation in the number of thrips individuals caught did not show any significant differences in relation to the petunia variety (f=1.301, p=0.226>0.05).

Table 3. Average number of thrips (±SE) specimens in relation to petunia varieties

Petunia varieties	N*	Mean	Std. Deviation	95% Confidence Interval for Mean		Min. *	Max. *	D'*
				Lower Bound	Upper Bound			
Tropical	18	3.6667	4.39251	1.4823	5.8510	0.00	14.00	0.267
Beautical Mix 2	18	2.7222	3.17723	1.1422	4.3022	0.00	11.00	0.246
Calipetite Mix 1	18	1.7778	2.62467	0.4726	3.0830	0.00	7.00	0.271
Calipetite Mix 2	18	3.7778	4.02281	1.7773	5.7783	0.00	14.00	0.199
Contrast MIx 1	18	2.7778	3.65506	0.9602	4.5954	0.00	12.00	0.216
Purple Picotee	18	1.6667	2.80755	0.2705	3.0628	0.00	8.00	0.209
Double Red	18	3.3889	4.81589	0.9940	5.7838	0.00	17.00	0.247
Cream Picotee	18	0.8889	2.16629	-0.1884	1.9662	0.00	9.00	0.053
Cherry Pop	18	1.6667	2.40098	0.4727	2.8606	0.00	7.00	0.204
Bicolor Yellow Red	18	2.5000	3.79241	0.6141	4.3859	0.00	11.00	0.330
Orange Bouquet	18	2.8333	4.34200	0.6741	4.9926	0.00	11.00	0.298
Pink Bouquet	18	1.3333	2.72246	-.0205	2.6872	0.00	9.00	0.222
Total	216	2.4167	3.53882	1.9421	2.8913	0.00	17.00	0.263

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	176.500	11	16.045	1.301	.226
Within Groups	2516.000	204	12.333		
Total	2692.500	215			

* N - number of samples analysed; Mean - mean number of specimen/ sample; Min. - minimum number of specimens/sample; Max - maximum number of specimen/ sample; D' - Simpson's Index

Possible causal relationships between thrips and petunia plants were revealed by Simpson's index analysis using the observed thrip-flower associations in petunia (n = 2.4167), which we considered as potential explanatory variables (Table 3 and Figure 2). for the abundance of the major thrips species in petunia.

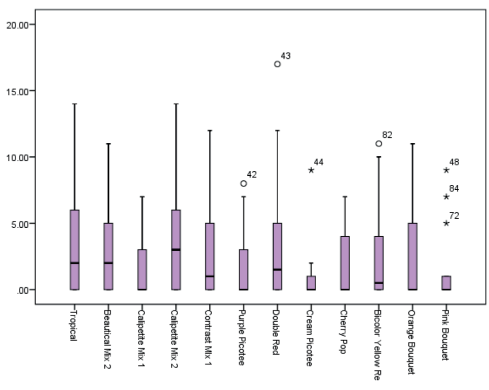

Figure 2. Graphical representation of the average thrips number related to petunia variety

All six thrips species were observed in all 12 *Petunia hybrida* varieties studied, but at different frequencies, suggesting that thrips prefer different petunia plant varieties. The value of Simpson's D ranges from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity, so the larger the value of D, the lower the diversity (Mound, 2018). The results of calculating the diversity index (D) of thrips species were found to be 0.263 (D close to 0). These results suggest that thrips diversity is moderate to high in examined petunia varieties. The highest diversity values were recorded for 'Cream Picotee' (D= 0.053) and the lowest for 'Bicolor Yellow Red' (D= 0.330). The most suitable host varieties or those with moderate diversity of thrips pests were 'Calipetite Mix 2', 'Cherry Pop' and 'Purple Picotee'.

A further - and most important - aim of the research was to highlight the colour preferences of the different species of thrips. Coloured sticky traps are one of the most useful and effective tools available for the collection of insect pests, including thrips. Colour attractiveness and the rate at which thrips are captured depend on the type of plant (Kirk, 1984) and the colours of the traps (Beckham, 1969; Cho et al., 1995). In 2023, more than 48.93% of the specimens were attracted by the yellow traps, the remaining were attracted by orange - 17.6% and blue - 33.47%.

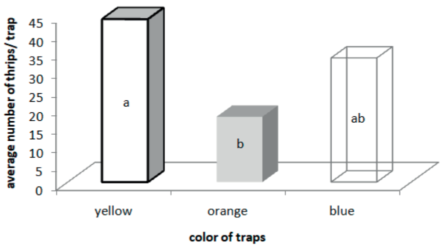

Figure 3. Average number of thrips specimens caught by the colour traps
(Columns denoted by different letters are significantly different at a significant level $p < 0.05$)

Although the yellow traps attracted the highest number of specimens ($F=39.398$, $p=0.000<0.05$), followed by the blue traps, significant differences were observed depending on the colour of the trap; these differences were found between the yellow-blue ($p=0.000$) and the yellow-orange ($p=0.000$) traps. The blue traps and the orange traps attracted the specimens to a certain degree in different ways. No significant difference was found between the attractions of the two colours (Figure 3). Thrips show a greater phototactic response to yellow colour compared to other colours (Thongjua et al., 2015). The effective attraction of thrips to yellow traps in this study is in line with studies conducted by researchers on a number of crops (Jenser et al., 2001; Vîrteiu et al., 2022).

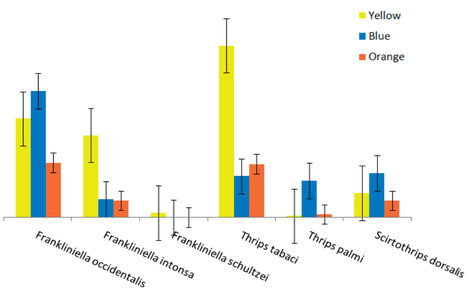

Figure 4. Mean proportions (± SE) of different thrips species caught on petunia plants with coloured sticky traps

Yellow sticky trap at 15 cm attracted more thrips, including *Thrips tabaci*, the species considered to be an economically important pest of ornamentals and vegetables. The greatest attraction of the thrips to the yellow sticky trap was observed for two other species of thrips: *Frankliniella intonsa* and *Frankliniella schultzei* (Figure 4).

Other reports (Cho et al., 1995; Elekçioğlu, 2013) have also shown that the average number of adult thrips caught in blue sticky traps was usually higher than that in yellow sticky traps. Three species preferred blue sticky traps: *Frankliniella occidentalis*, *Thrips palmi* and *Scirtothrips dorsalis*. Our results under conditions of relatively high densities of *F. occidentalis* are consistent with those of Robb (1989) and Seo et al. (2006), who found that blue traps attracted more *Frankliniella occidentalis* than other colors (like green or white) in an ornamental glasshouse. The present research is partially similar to that of Cho et al. (1995), who indicated that blue sticky traps were preferred by *Frankliniella occidentalis* and *Thrips tabaci*, followed by white sticky traps; and contrasts with the results obtained by Hoddle et al. (2002), who indicated that white sticky traps were preferred by *Frankliniella occidentalis*. The orange traps were the least effective.

CONCLUSIONS

With 6 species identified, the study revealed a high taxonomic diversity.

The most abundant species were *Frankliniella occidentalis* and *Thrips tabaci*.

Structural indices show a dynamic fauna, most species collected being polyphagous.

The *Thripidae* fauna hosted by the petunia inflorescences was dominated by species that are typically floriphagous and, at the same time, dominated by species that are mesophilic.

'Calipetite Mix 2' variety was preferred by thrips among the petunias studied, at the same time, 'Cream Picotee' variety was the least affected by thrips species.

The yellow proved to be the most reliable colour for monitoring thrips populations.

Orange and blue could be used with weaker, but still reasonable effectiveness.

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THE USE OF NATURAL MATERIALS IN THE DESIGN OF URBAN PLAYGROUNDS: A SUSTAINABLE APPROACH IMPORTANT FOR THE COMMUNITY

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Abstract

Play is essential for the harmonious development of children. Playing outdoors brings with it a multitude of well-known benefits. The presence of playgrounds in the urban environment becomes all the more important as children`s connection with the natural environment becomes increasingly precarious. The appearance of standardized play equipment unfortunately omitted the primary need of children to discover the surrounding environment through challenges. The stereotyped design practiced in the arrangement of play spaces reduces the possibilities of developing a child`s personality and expressing his creativity. The present study aims to address the importance of designing play spaces with a natural concept that will contribute positively to the cognitive development, creative thinking, flexibility, and adaptability of children to various situations. The paper proposes solutions for their correct utilization by providing them with equipment made of carefully selected natural materials and integrated in an appropriate compositional environment. The final goal is to develop some good practice models that can later be implemented in the network of urban spaces intended for children in the city of Iași.

Key words*: community, natural materials, urban playgrounds.*

INTRODUCTION

In the field of humanities, such comprehensive and deeply explored themes as life, death, love, war, freedom, or human identity often pose challenges in synthesizing the essence of the researched subject. The same holds true for the concept of "play." The multivalence of the phenomenon arises from the subtlety of how play unfolds, seemingly emerging abruptly at any given moment and dissipating just as swiftly (Henricks, 2008).

Within the play action, a constant interplay of varied behaviors and human connections is observed, making it challenging to establish behavioral patterns with fundamental roles in defining the phenomenon of play.

Throughout historical development, psychologists and educational theorists have tended to determine the nature and value of play, thus giving it a clearly defined role in the structure of human existence (Huizinga, 1955).

Swiss psychologist and philosopher Jean Piaget (1962) proposed a distinct concept for the act of play, explaining it as a natural absorption phenomenon characterized by children's effort to infuse with their surrounding environment their own concepts and behavioral values.

Thus, during the game, children conceive their own plans, apply them in various situations, and experience a feeling of fulfillment at the moment of discovering the effectiveness of these structures.

Through play, the child discovers control and self-direction and builds the necessary skills and confidence to use those skills in various situations (Henricks, 2008).

One of the most significant places with a major impact on the physical and mental development of children is the outdoor playground.

At an overall examination, at an early stage, this aspect can be inadvertently omitted, but following a thorough research, it is noted that urban playgrounds represent a complex element, an essential component of the community (Shackell et al.,2008).

In the specialized literature, the importance of playgrounds and their contribution to the physical and cognitive development of children has been highlighted countless times (Piaget,1962; Huizinga 1955).

The starting point for the presented study derives from the analysis of the trends of recent years regarding the accelerated evolution of virtual games, which attract the attention of children from an early age, so that they devote a considerable part of their time to the virtual world at the expense of exploring outdoor activities.

In parallel, the analysis of children's playgrounds as a component of the urban territory is particularly relevant due to the accelerated urbanization process. Although, on a daily basis, we witness innovative approaches to the planning and design of urban spaces, looking from an opposite perspective, it can be said that the evolution process of the urban environment sometimes shows such an accelerated speed that the aspect of the need to create a space for development, socialization, and fun for children is omitted. The children's natural play environment thus progressively acquires a segmented aspect, inadequate to the demands of the adjoining spaces.

From the perspective of good practices, it can be seen that most of the playgrounds in the city of Iași do not meet necessary standards, and the lack of clear legislative regulations regarding their arrangement accentuates this situation. (design requirements). The existing equipment is presented as repetitive modules that are constructively similar but lacking in character and uniqueness. The standardization of playgrounds facilitates the emergence of an unproductive environment for the development of children's intellectual abilities.

It is substantial that the spaces where children spend their time are associated with remarkable images and experiences that will facilitate familiarization with various life situations. At the same time, they contribute to the formation of social skills by being a space where groups of children of various ages can meet and communicate. Through play, children assimilate new social and cultural standards, discovering a vast spectrum of inner experiences (Huizinga, 1955).

The value of this study is all the more notable, as it focuses, in a significant proportion, on the importance of natural elements incorporated in the design of play spaces.

Playing in nature is characterized as a process of assimilation in which children make a joint effort to improve their physical skills and imagination, a fact that leads to a deeper knowledge of the surrounding environment.

The physical facilities of the playground are recommended to be designed in such a way as to allow direct contact with nature in the child's daily routine. Those children who have no contact with the natural environment will fall into the category of individuals who will never perceive human dependence on the world around them (Moore, 2014).

The authenticity of the study consists in the examination of design principles and the design of urban play complexes in the city of Iași, with the ultimate goal of creating outdoor urban spaces where children of any age can socialize through play and develop new skills by interacting with a wide range of natural elements and materials.

MATERIALS AND METHOD

Three playgrounds were identified and inventoried as the method of study. The respective spaces were selected due to their location in some of the busiest neighbourhoods of the city: Nicolina (Figure 1), Alexandru cel Bun (Figure 2), and Copou (Figure 3).

Figure 1. The playground located in the Nicolina neighborhood, Iași

Figure 2. The playground located in the Alexandru cel Bun neighborhood, Iași

Figure 3. The playground located in the Copou neighborhood, Iași

A considerable difficulty in the design of current spaces for children in Iași is the fact that most of them are intended for children, aged between 2 and 6. Although the rules officially established for the use of these spaces states their use by children between the ages of 2 and 14, in reality, they are aimed at those children who are at an early stage of life and are just beginning to explore their environment. Following the analysis of these spaces, namely the field inventory of the equipment, the dominance of a central static structure made of materials such as metal and plastic, characteristic of most children's playgrounds, was observed. This type of structure fragments the surface intended for the game, thus there is only one area of interest; the adjoining space is not used, resulting in a compositional disagreement.
To propose suitable design solutions, the next step involved examining specialized literature regarding best practices for using natural materials in playground arrangements.
One of the basic roles of the design of these types of spaces is to actively contribute to shaping the child's personality, encouraging him to express his individuality in the social environment. In order to achieve the given goal, it is necessary that the space, through its design and composition, incite action.
To exemplify this concept, a new design is proposed for the playground located in the Alexandru cel Bun neighborhood, one of the busiest areas in Iași. It has a relatively large space but is improperly maintained, practically unusable.

RESULTS AND DISCUSSIONS

Through the given proposal, the study seeks to support the idea that the use of design tools, which are meant to solve the users' problems, in an educational space, instead of applying standard methods, will contribute to the formation of children's consciousness and their ability to think and solve various problems in the community.
Sculptor Simon Nicholson, in his work in 1971, proposed the idea that materials that can be manipulated and reconstructed create more possibilities for creative involvement than static pieces (Nicholson, 1971).
Handling things, managing possibilities, and taking the risks that come with them teaches children how to act in various situations. This is a core component of the growing process, and play is one of the most significant ways in which they sharpen this essential skill. (Frost & Klein, 1979).
In his theory, Jean Piaget (1962) formulated the idea that, in order to discern the sense and importance of anything, the child must realize it independently, assigning it a new meaning, so that during the process he evolves into a personality capable of creativity, not just repetition (Piaget,1962).
The inclusion of natural elements in the evolution of children's personalities is a fundamental part of the relationship of future generations with nature. (Wellls & Lekies, 2006)
In order to ensure the success of the given link, it is a priority to find new ways to reintegrate vast natural experiences into the lives of the little ones. Such actions will contribute to strengthening the framework for the development of a generation of energetic and physically healthy children, as well as increasing the affection for nature and the perception of the interdependence between humans and a healthy ecosystem (Chawla, 1998).
Thus, the proposed design wants to encourage spontaneous research activities inside a play space by using natural materials such as stones of various sizes, tree trunks, logs, wood slices, branches, bark, sand, and furniture made of wood.
Sand is one of the most versatile materials because, due to its structure, it can be shaped in

countless ways, providing opportunities for creative and sensory play (Moore, 2014).
The sand area can be delimited within the compositional space by logs, to form a spatial boundary and for more suitable maintenance (Figure 4).

Figure 4. Exemplifying the use of sand

Sand areas are spaces where several children of different ages can interact simultaneously. Although sand is one of the main elements that contribute to the development of preschoolers' fine motor skills (Jarret et al., 2010), it also easily attracts children aged 6-12, as it offers a wide range of play opportunities in various social settings. (Frost and Woods, 1998). Other items with high play value are stones (Figure 5) and tree trunks (Figure 6).

Figure 5. Exemplifying the use of stones and boulders

Figure 6. Exemplifying the use of stones and tree trunks

These elements contribute to the development of the sense of touch, offering the chance to analyze the different textures of natural materials and the possibility of acquiring such skills as climbing small obstacles (Figure 7) or swinging on rows of logs (Figure 8).

Figure 7. The use of small obstacles for climbing

Figure 8. The use of rows of logs

A set of stones is a simple technique for creating a natural play structure. The use of locally sourced stones will add an authentic element to the landscape, being not only a cost-efficient source of equipment but also a basic piece for implementing free play and remodeling the ensemble into infinite patterns, depending on the children's imagination.
An interesting play space will have places that encourage exploration and that offer certain surprises without having full visibility from the moment of entering the respective setting. Grass mounds and slopes bring that element of surprise to the landscape while producing the illusion of a more expansive space. These elements with varied slopes are fun for children of a wide age range and can be used in plain form or integrated with fixed structures, which will increase the value of play and social interactions (Figure 9) (Moore, 2014).
They add complexity to the space, allowing the execution of actions such as rolling, dragging,

and sliding, which stimulate the perception of the sensations of movement offered by the human body.

Figure 9. Exemplifying the use of grass mounds and slopes

In terms of vegetation, the use of elements such as trees, shrubs, perennials, and grass accentuates the natural character of the space and facilitates its integration into the natural setting.
Vegetation elements must be perceived as play elements (Table 1), not just decoration, so the design must be dictated by the methods through which the vegetation elements can be integrated into the play and not the other way around (Shackell et all.,2008).

Table 1. Vegetation elements proposed for use in playgrounds

Element name	Play feauture
Salix matsudana f. *tortuosa* (Vilm.) Rehder	Unusual, twisted stems
Salix alba L.	Stems can be used to make different play structures, like huts or tunnels.
Pinus sylvestris L.	Needles and pine cones
Betula pendula Roth	Bark and catkins
Cercidiphyllum japonicum Siebold & Zucc.	Leaves and fragrance
Ornamental grasses	Can be used for sensory play, and the seed heads are play elements.
Perennial plants, exemples: *Stachys byzantina* K.Koch *Zinnia elegans* L. *Tagetes* L. *Bergenia cordifolia* (Haw.) Sternb.	Natural play materials

Plantings can constitute screening, protection, and comfort systems, adding interest to the space through seasonal visual diversity. Vegetation enriches the child's daily life through varied experiences of smell, color, and texture, and the element of grass is the most versatile material that can be used in a play space.
One of the main aspects of the study is the integration of activities intended for various age groups in playgrounds, an aspect that is lacking in the current urban conditions. One of the reasons that leads to the presence in current playgrounds of only static equipment well anchored in solid surfaces is the fear of playground destruction. This fear may unintentionally represent the starting point for the orientation of playground equipment only towards young users due to not attracting the adolescent age group, which is considered more problematic in society. Consequently, in the city of Iași, there is no urban space intended for teenagers. In such circumstances, it cannot be unexpected that the feeling of not belonging within the community and the manifestation of violent outbursts can lead to vandalism.
Taking into account the above, a zone for teenagers was also introduced in the proposed design, characterized by a space for playing basketball and adjacent places for meeting and socializing (Figure 10).

Figure 10. Exemplifying the space intended for teenagers

The proposed design is an attempt to combine several age groups. Although being systematized according to these, the aim of the project was to create a permissive concept regarding which activities and which age categories are or are not allowed in a certain area of the space, allowing users a high degree of flexibility regarding the use of the play space.

CONCLUSIONS

In a child's development process, play is essential. This research was initiated out of a desire to encourage the creative use of playground equipment and to emphasize that they are interconnected components in a design proposal and not its main features.

Much of the equipment present in play spaces is designed to be easy to reproduce, but the play opportunities that challenge the child to explore are missing.

The study does not recommend eliminating prefabricated playground equipment but emphasizes the need to change the community's perception of the importance of the materials used in their design.

Thus, the proposed natural elements - wood, sand, logs, and vegetation elements - contribute to increasing the degree of creativity and guiding children towards autonomous behavior, which is carried out without an external goal or reward and is a fundamental tool for sustainable development.

The implementation of this idea at the level of the communities will increase their awareness as well as that of the governing bodies regarding the importance of developing playgrounds for children and equipping them with installations to stimulate curiosity and knowledge of the environment in a healthy way.

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THE MINERAL COMPOSITION OF FLAX, CHIA AND HEMP SEEDS BASED ON X-RAY FLUORESCENCE ANALYSIS

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Abstract

Due to the fact that flax seeds contain the highest amount of essential fatty acids from plants, they are increasingly used as a functional food. Chia seeds are an important source of antioxidants that have beneficial effects on the human body. Hemp seeds are recommended for consumption because they are an excellent source of protein, vitamin E and minerals, and contain all the essential amino acids. The aim of this study is to assess the mineral profile of three types of seeds: flax, chia and hemp. The determination of minerals was made by X-Ray Fluorescence Analyzer. The seeds mineral fingerprints were obtained by using PAST and MVSP programs. The results of the analysis of 12 seeds assortments reveal that these are rich in phytonutrients and minerals (K, Ca, Fe, Mn and Zn). Chia seeds stand out for the highest content of calcium (32.26 g/kg dry weight), while hemp seeds have the greatest content of iron (0.88 g/kg dry weight).

Key words: *superfoods, XRF, nutrients, mathematical model.*

INTRODUCTION

Flax (*Linum usitatissimum* L.), chia (*Salvia hispanica* L.) and hemp (*Cannabis sativa* L.) are known as superfoods due to their nutritional properties (Barsby et al., 2021; Bordean et al., 2021; Montero et al., 2023, Kraska et al., 2016).
Flaxseeds have an exceptional nutritional value because contains α-linolenic acid, proteins, dietary fiber and phytoestrogens, therefore are used in food industry for obtaining functional foods as breads, cakes, cereal bars (Bhardwaj et al., 2020; Katare et al., 2012; Khalifa et al., 2011).
Chia seeds contains high amounts of ω-3 fatty acids, dietary fibre, gluten free proteins, vitamins, minerals polyphenolic compounds (i.e., gallic and p-coumaric acids), quercetin, kaempferol and antioxidants (da Silva et al., 2017; Ullah et al., 2016).
Cannabis sativa L. is a species in which the level of tetrahydrocannabinol (THC) is very low. EU regulations are limiting the amount of THC in hemp.
The area cultivated with hemp has increased in EU from 20.5 ha in 2015 to 33 ha in 2022 (an increase of 60%). Hemp seeds are an excellent source of protein with good digestibility, vitamin E and minerals, and contain all the essential amino acids (Żuk-Gołaszewska et al., 2018).
Hemp seeds, which are also exceptionally nutritious, are rich in three polyunsaturated fatty acids: alpha-linolenic acid, linoleic acid, and gamma-linolenic acid (Montero et al., 2023).
Hemp is also a good source of antioxidants (bioactive peptides) and phytochemicals (polyphenols and sterols) that are useful in supporting health (Rizzo et al., 2023).
Minerals are essential for the correct functioning of the body, most of them being involved in metabolism, the balance of water in the body and the health of the bone system. All three types of seeds are recognized for their high potassium and calcium content (Barsby et al., 2021).
The aim of this study is to assess the mineral profile of these three types of seeds, using the XRF spectroscopy.
Energy dispersive X-ray fluorescence, called XRF, is a fast, non-destructive method of measuring the elemental composition of a material.

MATERIALS AND METHODS

The study was performed on 12 types of seeds available on the Romanian market, four assortments of each type of seeds:

- flax (S1-S4);
- hemp (S5-S8);
- chia (S9-S12).

In our study we used the nutritional parameters (lipids, carbohydrates, proteins, fibers) mentioned on the labels of the seeds assortments.

Determination of samples mineral profiles was made by X-Ray Fluorescence using Hitachi X-MET8000 portable Spectrometer.

In order to analyze the mineral content, the samples were dried until constant weight, and then grounded.

The results are expressed in ppm (mg/kg dry weight).

The graphical fingerprints of the seeds based on mineral composition were obtained using PAST (Hammer et al., 2001) and MVSP programs.

RESULTS AND DISCUSSIONS

In Table 1 are presented the nutritional parameters of seeds samples, as they are mentioned on the products labels, expressed in g/100 g product.

Lipids content is high for all seed samples ranging between 30.2-50 g/100 g.

The protein highest content corresponds to the S8 sample (hemp seeds) (34.0 g/100 g) and the lowest to the S3 sample (flax seeds) (15.2 g/100 g).

Table 1. Nutritional parameter of seeds samples

Seeds samples	Lipids (g/100 g)	Carbohydrates (g/100 g)	Proteins (g/100 g)	Fibers (g/100 g)
S1	42.0	29	18	24.2
S2	42.1	1.5	18.2	27.3
S3	41.0	10.4	15.2	26.9
S4	30.9	0.0	24.4	38.6
S5	50.0	4.7	30.0	7.75
S6	30.2	3.4	20.0	33.9
S7	41.0	11.0	30.0	9.3
S8	48.0	3.1	34.0	6.0
S9	31.0	5.0	20.0	36.0
S10	31.0	42.0	17.0	34.0
S11	31.1	4.9	20.4	31.8
S12	30.8	43.8	15.6	37.7

The highest value of the fibers content corresponds to the S4 assortment (flax seeds) (38.6 g/100 g) and the lowest to the S8 assortment (hemp seeds) (6.0 g/100 g).

All the seeds taken in our study have high nutritional values due to the fact that they are rich in proteins and fibers (Table 1).

In Table 2 are presented literature data regarding flax, hemp and chia seeds minerals content.

Table 2. Mineral's content of seeds (Literature data)

Element	Flax seeds (Range)	Hemp seeds (Range)	Chia seeds (Range)
K (g/kg)	5.8 - 19.2 (Kluza-Wieloch et al., 2020) (Khan et al., 2010)	4.63 - 28.2 (Mihoc et al., 2012)	4.07 - 7.67 (Kulczyński et al., 2019) (Barsby et al., 2021)
Ca (g/kg)	1.38 - 2.36 (Barsby et al., 2021) (Katare et al., 2012)	0.7 - 9.55 (USDA, 2016) (Mihoc et al., 2012)	4.56 - 6.71 (Kulczyński et al., 2019) (Vera-Cespedes et al, 2023)
Fe (g/kg)	0.02 - 0.24 (Kraska et al., 2016) (Khalifa et al., 2011)	0.001- 2.4 (Callaway et al., 2004) (Mihoc et al., 2012)	0.07 - 0.145 (USDA, 2016) (Barsby et al., 2021)
Mn (g/kg)	0.015 - 0.04 (Kluza-Wieloch et al., 2020) (Kraska et al., 2016)	0.06 - 0.11 (Mihoc et al., 2012)	0.027 - 0.059 (Kulczyński et al., 2019) (Vera-Cespedes et al., 2023)
Zn (mg/kg)	43.7 - 78 (Barsby et al., 2021) (Kluza-Wieloch et al., 2020)	42 - 94 (Mihoc et al, 2012)	46 - 52 (Kulczyński et al., 2019) (Vera-Cespedes et al., 2023)
Cu (mg/kg)	2.31 - 25.6 (Khan et al., 2010) (Kluza-Wieloch et al., 2020)	10 -12.2 (Mihoc et al., 2012) (Barsby et al., 2021)	9 - 17.7 (Kulczyński et al., 2019) (Barsby et al., 2021)
Ni (mg/kg)	1.2 (Barsby et al., 2021)	53.2 (Barsby et al., 2021)	1.8 (Barsby et al., 2021)

In Table 3 are presented the minerals content of flax, hemp and chia seeds, analyzed in the laboratory by FRX method.

We observe that chia seeds stand out for the highest calcium content (32.26 g/kg dry weight) and hemp seeds for the highest iron content (0.88 g/kg dry weight).

Table 3. Mineral content of seeds analyzed in the laboratory

Element	Flax	Hemp	Chia
	Range	Range	Range
K (g/kg)	26.3 - 35.9	26.57 - 36.67	21.85 - 27.25
Ca (g/kg)	8.85 - 11.7	2.79 -3.9	23.71 - 32.26
Fe (g/kg)	0.49 - 0.64	0.67 - 0.88	0.46 - 0.52
Mn (g/kg)	0.12 - 0.22	0.4 - 0.46	0.38 - 0.55
Ba (g/kg)	0.122 - 0.13	0 - 0.15	0 - 0.158
Zn (g/kg)	0.215 - 0.27	0.23 - 0.45	0.16 - 0.25
Sr (mg/kg)	12 - 23	0 - 40	65 - 137
Cu (mg/kg)	74 - 86	62 - 88	74 - 94
Rb (mg/kg)	8 - 14	7 - 26	22 - 43
Ni (mg/kg)	0 - 23	0 - 23	0 - 27

Correlation between experimental laboratory and literature data

Using the average data of the mineral content presented in Tables 2 and 3 and applying linear correlation r, we can observe that the literature data show a strong positive correlation with the laboratory data for flax seeds (0.92522) and chia seeds (0.87914) and a positive low correlation for hemp seeds.

Figures 1, 2 and 3 show the correlation between the mineral content quantified in laboratory for flax, hemp and chia seeds and the literature data.

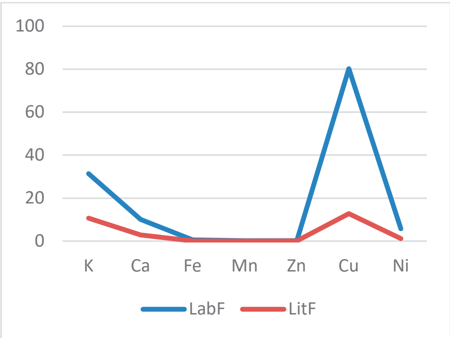

Figure 1. Graphical representation of values of flax seeds mineral contents obtained in the laboratory and those available in literature

(Legend: LabF = Flax mineral content analysed in the lab; LitF = Literature data for flax seed mineral content)

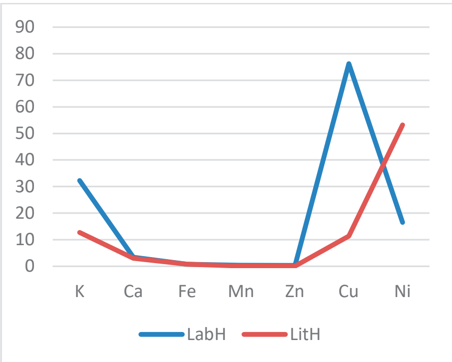

Figure 2. Graphical representation of values of hemp seeds mineral contents obtained in the laboratory and those available in literature

(Legend: LabH = Hemp mineral content analysed in the lab; LitH = Literature data for hemp seed mineral content)

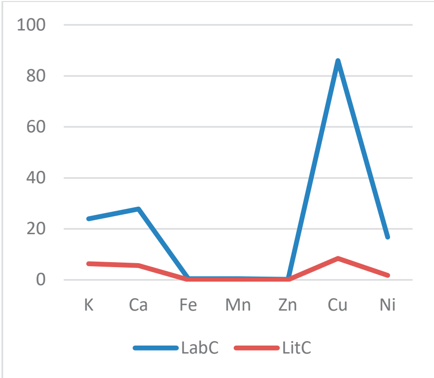

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Figure 3. Graphical representation of values of chia seeds mineral contents obtained in the laboratory and those available in literature

(Legend: LabC = Chia mineral content analysed in the lab; LitC = Literature data for chia seed mineral content)

The Figure 4 provides a graphical representation of how the data points (in this case, the seed samples) are grouped into clusters based on their similarities.

If we apply the Cluster analysis based on Paired group algorithm and correlation as similarity measure, we observe that the correlation coefficient is high (0.8633).

The correlation coefficient indicates the strength and direction of the relationship between the variables being compared. A high correlation coefficient suggests a strong positive linear relationship between the paired groups.

Based on the formed clusters chia seeds (green, S9-S12) presents a different profile compared to hemp (blue, S5-S8) and flax (red, S1-S4) (Figure 4).

This observation suggests that there are significant differences between the characteristics or attributes of these seed types. Based on the results we can observe that chia seeds have distinct characteristics that set them apart from both hemp and flax seeds.

This could be valuable information for further research, product development, or market positioning in areas such as nutrition, agriculture, or food science.

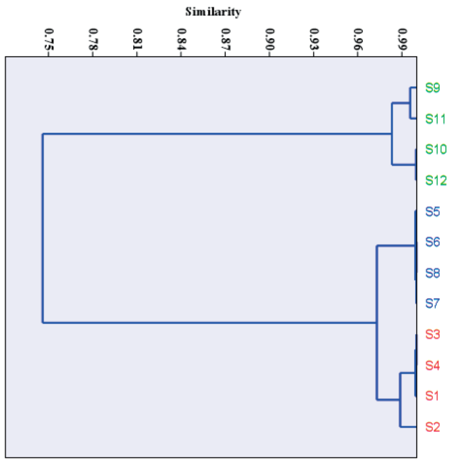

Figure 4. Cluster analysis

Legend: red color = Flax seeds (S1-S4), blue color = hemp seeds (S5-S8), green color = chia seeds (S9-S12)

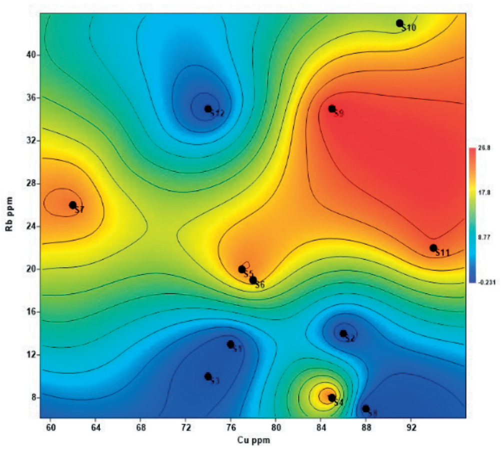

Figure 5. The spatial interpolation

Legend: Flax seeds (S1-S4), Hemp seeds (S5-S8), Chia seeds (S9-S12)

The spatial interpolation is presented in Figure 5. The rubidium content is highest in chia seeds, and based on the spatial interpolation, we can group the samples after the geographical origin criteria.

So, the first group with the assumed same origin is formed by S4, S5, S6, S7 samples; the second group by S1, S2, S3, S8, S12 samples and the third group by S9 and S11 samples, which is correlated with the origins of seed samples mentioned on the labels. This suggests that the samples within each group share similarities in terms of their origin or characteristics that led to their clustering together. Figure 5 provides a visual representation of the spatial distribution of the seed samples, allowing for a better understanding of their geographical patterns and relationships based on the clustering analysis results.

Figure 6 shows a graphical representation of a General Linear Model (GLM) applied to pairs of minerals: potassium (K) and calcium (Ca), strontium (Sr) and manganese (Mn), and copper (Cu) and rubidium (Rb) and provides insights into the relationships between different mineral pairs (potassium and calcium, strontium and manganese, copper and rubidium) across various seed types, highlighting potential patterns or interactions in mineral uptake or utilization within the seeds.

Potassium (K) and Calcium (Ca) Relationship: the observation suggests an inverse relationship between potassium (K) and calcium (Ca) content, particularly noticeable in chia seeds. As the potassium content increases, the calcium content decreases. The trend is more prominent in chia seeds compared to other seed types (flax and hemp). This inverse relationship suggests that there might be some form of competition or antagonistic interaction between potassium and calcium uptake or utilization in the seeds, leading to a negative correlation between their concentrations.

Strontium (Sr) and Manganese (Mn) Relationship: the observation indicates a positive relationship between strontium (Sr) and manganese (Mn) content in chia and hemp seeds. As the content of strontium increases, the content of manganese also increases for these seed types. This trend is not observed in flax seeds, signifying that the relationship between strontium and manganese may vary among different seed types. The positive correlation between strontium and manganese suggests a potential association or similar uptake mechanisms for these minerals in chia and hemp seeds.

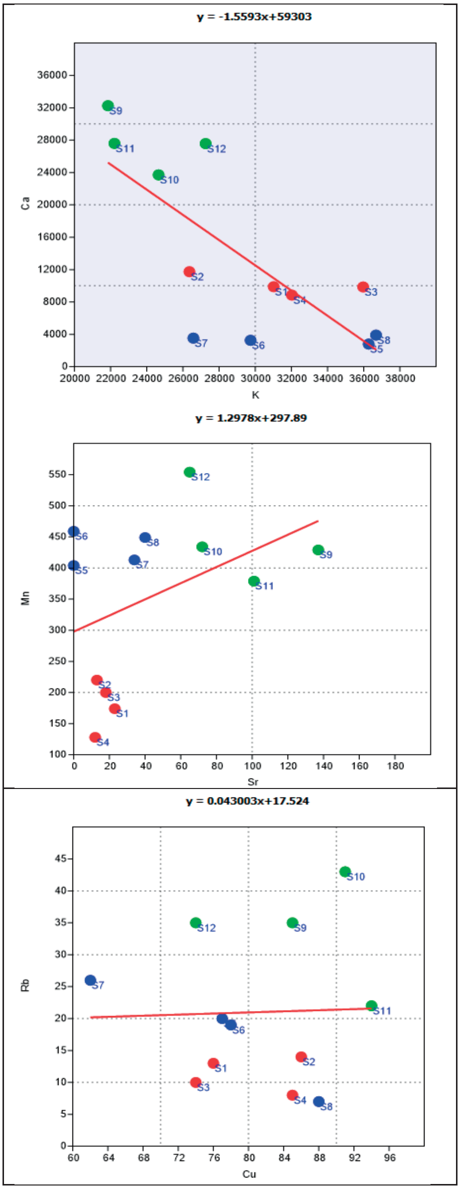

Figure 6. Representation of GLM (General linear model for K and Ca, Sr and Mn, Cu and Rb)

Legend: Red color = Flax seeds (S1-S4), Blue color = hemp seeds (S5-S8), green color = chia seeds (S9-S12)

Copper (Cu) and Rubidium (Rb) Relationship: the observation notes a slight increase in rubidium (Rb) content with increasing copper (Cu) content for all types of seeds. This suggests a positive correlation between copper and rubidium content across all seed types (flax, hemp, and chia). The slight increase in rubidium content with increasing copper content indicates a potential relationship or shared uptake pathways between these two minerals in the seeds.

Due to the various content of minerals of the investigated seeds, we have tried to identify the percent of minerals in case of associating different quantities of seeds (Figure 7).

Each seed type contributes varying amounts of minerals, and understanding these contributions can help in creating seed mixtures tailored to specific mineral needs.

Figure 7 visually represents the contribution of minerals from different seed mixtures and provides valuable information for consumers to make informed choices about seed mixtures based on their specific mineral needs, utilizing the unique mineral contributions of each seed type. It shows the percentage or proportion of various minerals contributed by each type of seed in different seed mixtures. The information suggests that consumers can choose seed mixtures based on their desired mineral content. For example: If a consumer needs a high content of potassium, they can choose a seed mixture with specific proportions of flax, hemp, and chia seeds to meet their requirements. Similarly, if they require a high content of manganese, they can select a different seed mixture with proportions adjusted to provide the desired mineral content.

Example Mixtures: for a high potassium content: a mixture consisting of 35% flax seeds, 37% hemp seeds, and 28% chia seeds. For a high manganese content: a mixture consisting of 15% flax seeds, 42% hemp seeds, and 43% chia seeds.

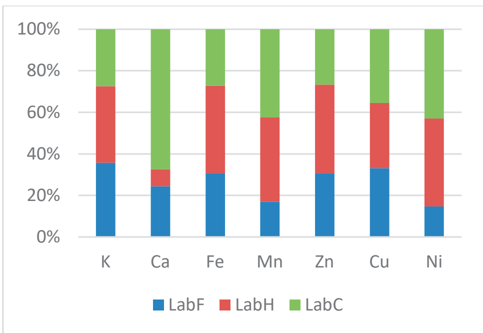

Figure 7. Contribution of minerals while using a seed mixture (Compositional mineral fingerprint)

(Legend: LabF = Flax mineral content analyzed in the lab; LabH = Hemp mineral content analyzed in the lab; LabC = Chia mineral content analyzed in the lab)

CONCLUSIONS

Our determinations using using the XRF spectroscopy revealed that chia seeds registered the highest content of calcium while hemp seeds have the greatest content of iron, observation being in accordance with the studies from literature data. But, using this method for minerals analysis we found higher quantities of K, Ca and Cu than those reported in other studies.

The observed relationships between minerals (such as K and Ca, Sr and Mn, Cu and Rb) differ across different seed types (flax, hemp, and chia). This suggests that the interactions between minerals may be influenced by the specific characteristics or properties of each seed type, including genetic factors, growth conditions, and metabolic processes.

The observed mineral interactions may have implications for agricultural practices and plant nutrition management. Farmers and agricultural researchers could explore ways to optimize mineral uptake and balance in seed crops through soil management practices, fertilization strategies, and crop breeding programs.

The observed mineral relationships could inform food formulation and nutritional strategies. Food manufacturers and nutritionists may leverage this information to develop seed-based products with optimized mineral profiles to meet specific dietary requirements or address nutritional deficiencies.

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WOOL-BASED FILTERS FOR THE RETAINING OF HEAVY METALS FROM INDUSTRIAL AND CONTAMINATED WASTEWATER

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Abstract

Water pollution occurs when chemical, physical, and biological elements exceed set limits. This phenomenon is primarily a result of various human activities, with industrial and municipal landfills playing a significant role in polluting the environment. Industries heavily dependent on chemicals, especially those that discharge metal-contaminated wastewater, pose a significant environmental concern because of the potential for bioaccumulation in organisms. The bioaccumulation of heavy metals in the food chain highlights the significance of this issue and its potential impact on consumer and public health. With various distinctive properties of α-keratin, wool-based filter systems can effectively manage heavy metal pollution. This paper will discuss the preliminary studies of the efficiency of wool-based filters for retaining heavy metals from different water sources, including wastewater, industrial water, and contaminated water.

Key words: *contaminated, filters, heavy metals, waste, wool.*

INTRODUCTION

Water is a crucial natural resource in any economic system since it serves as the basis for life and the growth of civilisation. Life on Earth would be impossible since water is intrinsically linked to life. People depend on water for their subsistence and progress because it is essential for agriculture, food, drink, and energy production (Afzaal et al., 2022).

Water constitutes around 75% of the human body and approximately 90% of the content of plants. It is indisputable that water is an essential component of life, and as people advance in their lives, their need for water becomes more demanding (Hassan Al-Taai et al., 2021).

Water was essential to the growth and prosperity of ancient human civilisations that progressed for millennia.

Records from the past make it abundantly clear that many civilisations flourished close to rivers. In addition to its fundamental significance, water plays an essential part in our world. It serves as the principal supply of fish, a vital food source all around the globe (Lin et al., 2022).

Additionally, water is used as a medium for linking diverse regions of the globe by utilising various techniques for marine transportation.

Pollution involves the harmful changes that may occur in several facets of the environment. It is an immediate result of human action, including both essential and industrial aspects, whether in whole or in part (Hassan Al-Taai et al., 2021).

The process commences with energy modifications, radiation levels, and unwanted biological, physical, and chemical changes in the biosphere, which is the habitat for all other animals. These alterations can influence the fragile ecological equilibrium, impacting the accessibility of sustenance, the purity of air and water, and the quality of agricultural commodities (Kaur et al., 2022).

A diverse array of environmental contaminants originates from several sources, each with its importance and influence. This phenomenon, known as environmental degradation, encompasses changes in the environment's quantity and quality, resulting in adverse effects on species and a reduction in the ecosystem's ability to support its production.

Drinking sediments and organic contaminants that reduce oxygen levels causes water pollution. These pollutants are emitted mainly by untreated urban sewers and industrial drains, exposing the environment to dangerous trace pollutants such as toxic compounds and heavy metals such as mercury, zinc, lead, and cadmium. Thermal pollution is a kind of water pollution caused by the discharge of power plants and industries and cooling water into water streams, causing increased temperatures and upsetting the natural balance of the aquatic ecosystem (Sonone et al., 2021).

Water pollution with heavy metals

Organic matter, nutrients, pharmaceuticals and personal care products, biocides, heavy metals, and nanoparticles are among the pollutants of significant concern.

Heavy metal ions are extensively discharged pollutants, and thus, they are of significant interest. Heavy metals are persistent, non-degradable, and non-soluble; therefore, they tend to bioaccumulate, increasing their concentration in living and aquatic organisms over time (Briffa et al., 2020).

Industrial activities often generate wastewater containing heavy metals, posing an increased environmental and health risk. Heavy metals such as lead, cadmium, mercury, and chromium are persistent and toxic substances that can accumulate in water and food chains.

Traditional methods such as chemical precipitation, ion exchange, and membrane filtration have cost, complexity, and efficiency limitations. Sustainable and cost-effective alternatives are therefore needed (Fu et al., 2011; Hezarjaribi et al., 2021).

Sheep wool has been found to be an excellent material for effectively retaining heavy metals from industrial wastewater because of its natural and renewable properties.

Wool's unique characteristics make it a favourable option for filtration systems (Enkhzaya et al., 2020).

This material's intricate structure features various functional groups, a significant surface area, and a remarkable ability to absorb substances.

Modifying wool chemically can improve its chemical and physical properties. Usually, these modifications are carried out on the mercapto (-SH) groups in sheep wool, leading to decreased disulfide bonds when exposed to alkaline reagents.

Sheep wool comprises 95% pure keratin, some hydrocarbons, and other compounds (Erdogan et al., 2020).

Sheep wool is a natural fibre obtained from keratin, a protein present in the hair of sheep. The chemical characteristics of sheep wool have a complex relationship linked to its partially crystallised protein polymer structure. This structure consists of a variety of amino acids that are linked together by peptide bonds. Sheep wool has specific characteristics that enable it to efficiently interact with and retain heavy metals in water pollution situations (Kuffner et al., 2012).

The working principle of sheep's wool filters

A better understanding of the working principle of sheep wool filters may be achieved by following the procedures that are provided below:

To complete the filtering process, the wastewater from industrial processes is passed through filters that include sheep wool.

While the water moves through the wool, heavy metal ions are absorbed into the fibres of the wool.

Ultimately, the wool acts as a sorbent, capturing the heavy metals and retaining them from the water, as we can see in the figure below (Alyousef et al., 2020).

Advantages of using sheep wool filters

Sheep wool filters provide several significant benefits compared to more traditional approaches to removing heavy metals (Figure 1) (McNeil et al., 2015).

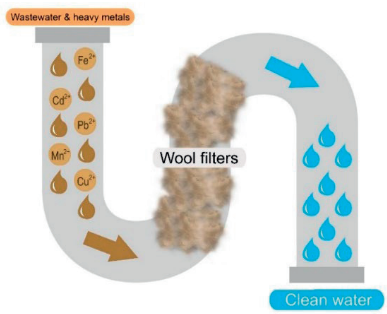

Figure 1. Working principle of wool filter

First, they are easily accessible and affordable, which is a significant factor in lowering treatment expenses.

Second, using of natural materials decreases the dependence on synthetic absorbents, decreasing the negative environmental impact. Wool's natural biodegradability also simplifies disposing of used filters, which provides an additional benefit.

The fact that sheep wool filters may be recycled and reused is another way they contribute to the product's sustainability (Doyle et al., 2021).

MATERIALS AND METHODS

For this analysis, we use sheep wool waste collected from a local farm, cleaned of contaminants or impurities and rinsed with distilled water.

The samples were analysed using the spectrophotometer Specord 200, (Analytik Jena, Jena, Germany). The heavy metal solution used was cadmium sulphate at 0.1 M concentration.

For its preparation, 2 grams of waste wool were cleaned and rinsed with distilled water.

A solution of 0.1 M/mL cadmium sulphate was applied to the wool in a total volume of 100 mL. After 30, 60, and 90 minutes, 10 mL of the sample was extracted and prepared for the spectrophotometric analysis, according to a method described by Ullah et al., 2011.

RESULTS AND DISCUSSIONS

Several scientific studies have been conducted to assess the efficacy of using sheep's wool filters to trap heavy metals (Enkhzaya et al., 2020).

The tests have yielded promising results, indicating significant retention rates for various heavy metals such as lead, copper, cadmium, and zinc (Figure 2) (Baltrenas et al., 2006).

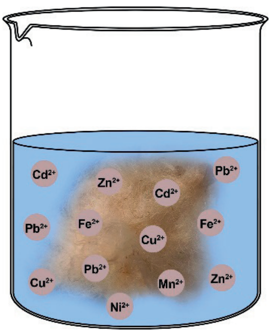

Figure 2. Heavy metals retention by the wool

Wool filters have interesting retaining capacities for heavy metals that make them highly suitable for various compositions of industrial wastewater (Babincev et al., 2020).

For this experimental study, we used a solution of cadmium sulphate as a heavy metal to demonstrate the efficacy of wool-based filters for retaining heavy metals.

The calibration curve for the heavy metal solution cadmium sulphate was generated by graphing the concentration against the sample's absorbance (Figure 3).

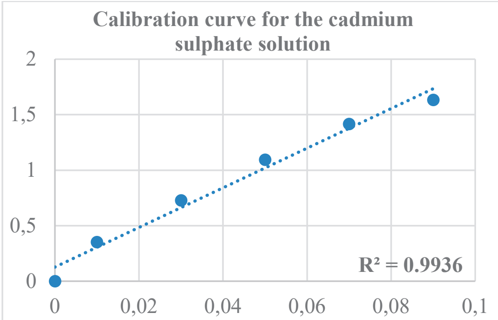

Figure 3. Calibration curve

The Table 1 illustrates the cadmium sulphate of sheep's wool filter over three separate measurements, 30, 60, and 90 minutes.

Table 1. Heavy metal solution in different contact time

Solution	Wool used	Contact time with heavy metal solution
Cadmium sulphate	2 g	30 min
	2 g	60 min
	2 g	90 min

These measurements are represented by vertical bars, each corresponding to a different value of retention capacity in percentage terms.

Based on the graphic data information, it is evident that sheep's wool filter demonstrates a significant capacity for retaining cadmium sulphate, with an efficiency that varies between approximately 70% and 77%.

As shown in Figure 4, the contact time between the wool and the heavy metal solution is an essential factor. After 60 minutes of contact, a higher retention is observed, while after 90 minutes, a decrease in retention (about 7% less) is registered.

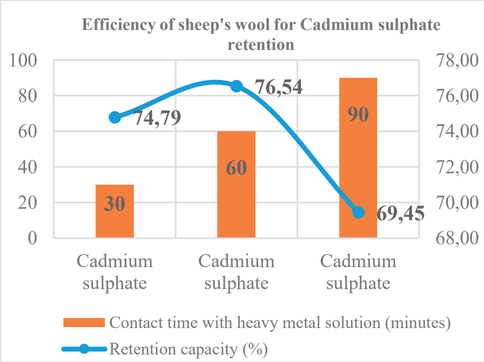

Figure 4. The efficiency of sheep's wool retaining for heavy metals

The measured maximum retention capacity is 76.54%. The ability to retain cadmium ions changes noticeably over different time intervals, indicating that wool's affinity for these ions might fluctuate during exposure.

The initial increase in retention capacity suggests a phase of accumulation, where the wool fibres demonstrate an increased ability to absorb cadmium ions.

The observed decline in the effectiveness of cadmium ion absorption may indicate a saturation threshold, at which point the wool's binding sites gradually become more inhabited.

When discussing these findings, it is important to consider the factors that could affect changes in retention capacity. These factors consider the rate at which the absorption process occurs, the point at which the wool's binding sites become saturated, and the possibility of cadmium ions being released back into the solution as time passes.

Including additional details about the specific time intervals during which these measurements were taken after the cadmium sulphate solution was administered will improve the overall understanding of the results.

Controlled experiments are essential to understand how these factors impact retention efficiency thoroughly. This could involve conducting experiments where one variable is deliberately changed while keeping all other factors constant.

This will allow for identifying the most effective methods to improve the efficiency of the filters.

CHALLENGES AND FUTURE PERSPECTIVES

Although the results obtained so far are encouraging, particular challenges are associated with sheep wool filters. More research is needed to evaluate the stability of the filters under different working settings, such as pH, temperature, contact time, initial metal concentration, and wool dosage, which significantly influence the absorption process. Furthermore, research on the regeneration and disposal of used wool filters is required to guarantee long-term usage (Hezarjaribi et al., 2021).

More research is needed to examine the possibilities of using new materials or changing wool fibers to increase their efficacy in retaining heavy metals.

To improve the retention efficiency of sheep's wool filters for cadmium sulphate or other heavy metal solutions, it considers the following approaches based on the following: (i) *Exposure time optimization*. The study reveals different levels of effectiveness at different time intervals. Investigating the optimal duration of contact to enhance absorption before reaching saturation could enhance retention efficiency;

(ii) *Modification of wool surface*. Through various treatments, the wool can acquire additional functional groups that can bind to heavy metals. This ultimately leads to increased accessible binding sites;

(iii) *pH adjustment*. The charge on wool fibers and heavy metal ions can be influenced by pH levels. Adjusting the pH of the solution could improve the bonding between heavy metal ions and wool, leading to better retention;

(iv) *Wool quantity*. Increasing the amount of wool in relation to the volume of the heavy metal solution could enhance retention by creating more binding sites;

(v) *Temperature control*. Optimizing the temperature to maximize the retention of heavy metal ions in wool can be beneficial, considering the impact of temperature on solubility and interaction;

(vi) *Regeneration of wool*. Investigating techniques to regenerate or purify the wool after it becomes saturated with heavy metal ions could make it reusable and maintain high retention rates over time (Condurache et al., 2022).

CONCLUSIONS

Sheep wool filers have shown considerable promise in efficiently retaining heavy metals from industrial wastewater activities. Sheep wool has remarkable attributes that make it a very effective filtering material and a notable ability to absorb heavy metals (Fu et al., 2011). Future studies should focus on enhancing the efficiency of wool modification procedures, incorporating sheep wool filters into wastewater treatment systems, and investigating combination filtering methods to improve the effectiveness of heavy metal removal (Gupta et al., 2021).

In conclusion, sheep wool filters have significant promise for efficiently eradicating heavy metals from industrial wastewater.

Due to ongoing technological advancements and research, these filters can significantly mitigate the environmental and health risks associated with heavy metal pollution in industrial activities.

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ASSESSMENT OF DECORATIVE QUALITIES OF *LYSICHITON CAMTSCHATCENSIS* (L.) SCHOTT. IN THE CONDITIONS OF THE RIGHT-BANK FOREST-STEPPE OF UKRAINE

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Abstract

People's natural attraction to beauty prompts them to decorate water bodies. As a decorative element of the water surface, aquatic flowering plants and the groups formed by them are of the greatest importance. The aim of our research was to find out the decorative properties of the introduced species Lysichiton camtschatcensis (L.) Schott. in the culture conditions of the "Sofiyivka" National Dendrological Park of the NAS of Ukraine. The decorative characters of plants was evaluated during 2013-2022, according to a scale that includes 20 main features that characterize the general appearance. The highest decorativeness of L. camtschatcensis was recorded according to the characteristics of the duration of flowering (15 points), the strength of the peduncle (10 points), the number of simultaneously open flowers in the inflorescence (12 points), the density (10 points) and the size (5 points) of the inflorescence, the diameter of the flower (5 points), shedding of flower petals (10 points), coloring (15 points) and falling of fruits (10 points). The total score of 141 points (amplitude 40-200) confirms the high decorativeness of L. camtschatcensis in culture conditions.

Key words: *introduction, score of decorativeness, signs of decorativeness, total assessment of decorative characters, water plant.*

INTRODUCTION

As a decorative element of the water surface, aquatic flowering plants and the groupings formed by them have significant importance. Professional selection of plants increases their longevity and the operational period of water bodies. The choice of appropriate plant material depends on the degree of decorativeness, which will enhance the charm and overall impression of the composition (Danilik, 2004).

Among vascular plants cultivated for ornamental purposes, representatives of the *Araceae* Juss family are quite popular. These are typically perennial herbaceous plants with rhizomes or bulbs. Their inflorescence consists of a spadix, almost always surrounded by a modified leaf called a spathe. In monoecious aroids, characterized by the presence of male and female flowers on the same inflorescence, the spadix is usually organized with female flowers located at the bottom and male flowers at the top of the inflorescence (Long et al., 2017).

The family *Araceae* belongs to the order *Alismatales* and includes about 144 genera and 3600 species (Boyce & Croat, 2018; Jiménez et al., 2019).

They are predominantly distributed in tropical and subtropical regions, especially in the tropics of the New World. Some species are widely used as indoor decorative and garden plants. However, the species diversity of decorative representatives still sparks discussions. For example, in China, the diversity of the *Araceae* family comprises 181 species belonging to 26 genera. Among them, only 20 species have aesthetic value. Therefore, introduced species are increasingly used in landscaping. For the past 30 years, approximately 120 species from 14 genera of exotic plants from the *Araceae* family have been cultivated in China (Long et al., 2017).

Studies on the decorative properties and prospects of using hydrophilic plants in water body landscaping in Ukraine have been conducted by R.M. Danilik (2004), V.P. Kucheryavyi and R.M. Danilik (1997), V.M. Holub and N.P. Holub (Holub & Holub, 2002; Holub, 2001), D.V. Dubyna (1982), T.P. Mazur (2000), M.Ya. Didukh and I.V. Chikov (2011), O.K. Red'ko and A.I. Kushnir (2013), G.A. Chorna (2006).

In particular, R.M. Danilik (Danilik, 2004) studied the hydrophilic vegetation cover in the ecological optimization of water ecosystems within the comprehensive green zone of Lviv city. The features of arrangement and landscaping of artificial and natural water bodies were analyzed by V.P. Kucheryavyi and R.M. Danilik (Kucheryavyi & Danilik, 1997). V.M. Holub and N.P. Holub (Holub & Holub, 2002; Holub, 2001) investigated the hydrophilic flora of the Prydniprovska Upland, decorative hydrophytes of the Right-Bank Forest-Steppe of Ukraine, and the prospects of their use in water body landscaping. In particular, they were involved in the introduction of rare species such as *Trapa natans* L. and *Salvinia natans* (L.) All. into the water bodies of the National Dendrological Park "Sofiyivka" of the National Academy of Sciences of Ukraine.

D.V. Dubyna (Dubyna, 1982) analyzed the species composition of hydrophilic flora, as well as distribution, stocks, biology, rational use, conservation issues, and species enrichment of the *Nymphaeaceae* family. T.P. Mazur (Mazur, 2000) and M.Ya. Didukh and I.V. Chikov (Didukh & Chikov, 2011) studied the taxonomic composition of native and introduced species and varieties in collections of aquatic and riparian plants. In particular, they researched life forms, allelopathic and bactericidal activity of plants, and studied the characteristics of seed and vegetative propagation, primarily representatives of the *Nymphaeaceae* family.

Red'ko O.K. and Kushnir A.I. (Red'ko & Kushnir, 2013), along with Ishchuk L.P. (Ishchuk, 2010a; 2010b), analyzed various groups of decorative plants, most suitable for landscaping water features depending on the zoning of the reservoir, its economic purpose and the structure of the surrounding landscape.

G.A. Chorna (Chorna, 2006) studied the flora of water bodies and marshes in the Forest-Steppe of Ukraine, the ruderalization of coastal habitats, as well as the floristic and cenotic features of mesotrophic marshes.

Among aroids, a group of decorative aquatic and semi-aquatic plants has attracted significant interest - *Calla palustris* L., *Lysichiton camtschatcensis* (L.) Schott., *Orontium aquaticum* L., *Pistia stratiotes* L., *Zantedeschia aethiopica* (L.) Spreng., which have long been cultivated as part of the collection of hydrophilic plants in the National Dendrological Park "Sofiyivka" of the National Academy of Sciences of Ukraine (Chikov, 2016).

One of the less common hydrophilic plants used in landscaping in Ukraine is the Far Eastern introduction *L. camtschatcensis*. In Japan, early in spring, right after the snow melts, this species creates an effect of white spots on marshes and in swampy alder forests due to the formation of a large white spathe (Fujita, 1997; Ohara, 1997).

Lysichiton camtschatcensis - is a large, sprawling, perennial herbaceous plant reaching up to 75 cm in height, with a thick, white, short, erect rhizome. The basal tufted leaves, numbering from 3 to 8, are gathered in a rosette, with a spongy petiole that is almost flat-convex in cross-section. The leaf blade is large, simple, entire-margined, ranging from elliptic to narrowly elongated, semi-blunt, narrowing near the base, with a thick midrib and lateral veins curved inwards at the tip, not reaching the edge. After flowering, the petiole with the leaf elongates to 40-80, sometimes 100 cm in length, and 15-30 cm in width. The flower stalk is 10-30 cm long and up to 2 cm thick, densely covered with a narrow tubular lower part of a white cover - a modified leaf, 8-15 cm high, transitioning to an elliptical or egg-shaped upper part, which, when fully unfolded, measures 10-19 cm in length and 6-17 cm in width, with a sharply pointed tip. The spadix is cylindrical, densely many-flowered (over 100 flowers), 4-8 cm long and 1-3 cm thick during flowering, and elongated to short-cylindrical, up to 12 cm in length and 5 cm in width during fruit formation. The flowers are bisexual, with 4 small tepals, rounded at the tip; 4 yellow stamens, with a conical-ovate style; berries are 2-seeded; endosperm is absent. Decorative period: April - July (Vegetation of Japan, 1988; *Lysichiton camtschatcensis*. Encyclopedia, Science News & Research Reviews., URL: https://academic-accelerator.com/encyclopedia/lysichiton-camtschatcensis).

According to taxonomic data from WFO (WFO, 2024. *URL: https://www.worldfloraonline.org/taxon/wfo-0000231604* 2024), *L. camtschatcensis* has several synonymous names, including *Aretiodracon camtschatcensis* (L.) A. Gray, *A. camtschaticus* (Spreng.) A.

Grey, *A. japonicum* A. Grey, *Dracontium camtschatcense* L., *D. camtschaticum* Spreng., *L. album* Makino, *L. camtschatcense* (L.) Schott, *L. camtschaticus* Schott, *L. japonicas* (A.Gray) Schott ex Miq., *Pothos camtschaticus* Spreng., *Symplocarpus camtschatcensis* (L.) Bong, and *S. kamtschaticus* Bong. These names, to some extent, reflect impressions from the external appearance and indicate the origin of this species.

The aesthetic features of *L. camtschatcensis* are also reflected in its folk names. For example, the general name "asian skunk cabbage" derives from the term "skunk cabbage," which is used for the entire genus *Lysichiton*, including *L. americanus* Hultén & H. St. John, known for its unpleasant odor. In Japanese, *L. camtschatcensis* is known as "mizubasho" (水芭蕉) due to its resemblance to Japanese bananas. This name evokes more poetic associations rather than an unpleasant odor. Names like "far eastern swamp lantern" or "Japanese swamp lantern" highlight the significant visibility of the white spathes even in darkness (source: https://academic-accelerator.com/encyclopedia/lysichiton-camtschatcensis). The hybrid *L. camtschatcensis* × *L. americanus* = *L.* × *hortensis is* also used for greening coastal wetlands. It is distinguished by larger size compared to the parent forms (*L. camtschatcensis*. Encyclopedia, Science News & Research Reviews., URL: https://academic accelerator.com /encyclopedia/lysichiton-camtschatcensis).

In natural conditions, *L. camtschatcensis* grows in forests in well-moistened areas, near streams, along forest clearings, and in marshy meadows. It looks particularly beautiful near streams, where its delicate white spathes are reflected in the water. In ditches on compacted poor soils, on overgrown areas, *L. camtschatcensis* forms smaller inflorescences and leaves.

L. camtschatcensis is common in mountainous regions along the coast of the Sea of Japan from the northeast to the southern part of Honshu Island, at around 35° north latitude. It also grows in the Khabarovsk Krai (Shantar Islands, mouth of the Amur and Ussuri rivers, northwest coast of the Tatar Strait), in the Magadan Oblast, on the Kuril Islands, the southern part of the Kamchatka Peninsula, and Sakhalin Island (Horikawa, 1992; Fujita, 1997; Vegetation of Japan, 1988).

In Japan, the distribution range of *L. camtschatcensis* closely overlaps with the range of *Alnus japonica* (Thunb.) Steud. The vegetation is represented by a marshy community of *A. japonica* and *Salix udensis* Trautv. & C.A. Mey, reaching heights of up to 10-11 meters with an admixture of *Fraxinus mandshurica* Rupr. var. *japonica*. The understory and shrub layers are sparse. *L. camtschatcensis* dominates in hygrophilous meadows. Herbaceous ground cover is composed of *Calamagrostis langsdorffii* (Link) Trin., *Carex pseudocuraica* L., *Carex rhynchophysa* C.A. Mey, *Cicuta virosa* L. and *Phragmites australis* (Cav.) Trin. ex Steud. (Fujita, 1997).

In the southern part of the Sakhalin and Hokkaido islands, as well as in the southern Kuril Islands, *L. camtschatcensis* is found in species communities with *Caltha fistulosa* Schipcz. and *Carex rhynchophysa* C.A. Mey. The canopy of *Alnus hirsuta* (Spach) Rupr. is usually higher than that of *Salix udensis*. In the herbaceous layer, a high projective cover is noted, consisting of a combination of species such as *Carex dispalata* Boott., *Filipendula camtschatica* (Pall.) Maxim., *Parasenecio hastatus* (L.) H. Koyama subsp. orientalis, and *Petasites japonicus* (Siebold & Zucc.) Maxim. The number of vascular plant species per 100 m^2 ranges from 23 to 30 individuals, while ground mosses reach up to 3 individuals. The tree canopy consists of 1-2 tiers, with the upper tier reaching heights of 9-10 meters and the lower tier reaching 6-7 meters. The crown density is about 55%. The shrub layer is almost absent (projective cover - 1%). Conversely, the herbaceous layer is well-developed, with a projective cover of about 100%, including ground mosses, which make up less than 1% (Vegetation of Japan, 1988).

MATERIALS AND METHODS

In connection with a person's subjective perception of the habits of plants, the problem of an objective comparative assessment of their decorative qualities arises.

The aim of our research was to determine the decorative properties of *L. camtschatcensis* as a promising species for landscaping water bodies in Ukraine.

The research was conducted from 2013 to 2022 in the water bodies of the "Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine. The habitat of *L. camtschatcensis* in the water bodies of the "Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine is shown in Figure 1. *L. camtschatcensis* was introduced in May 2013 from the upper reaches of the water body of the Botanical Garden of Ivan Franko Lviv National University (Catalog…, 2015), where this species grows in an area of about 100 m^2.

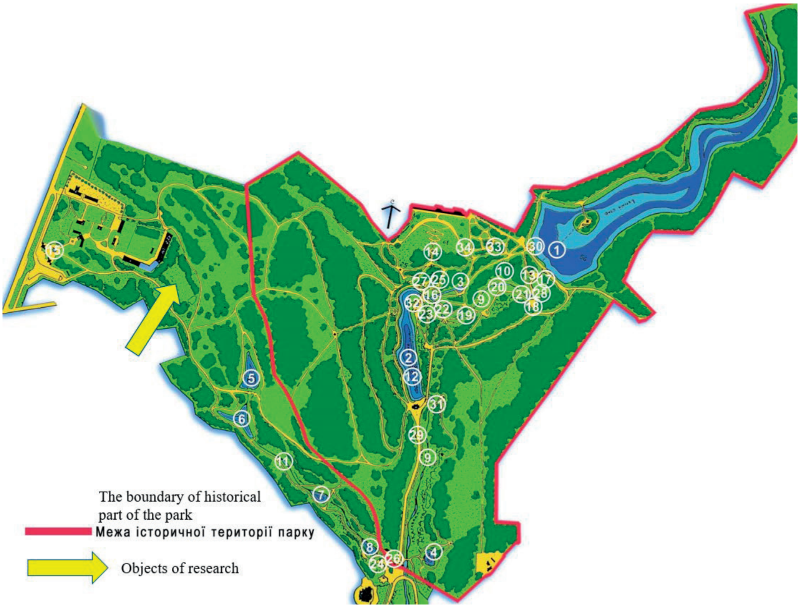

Figure 1. Map showing the placement of water bodies with *L. camtschatcensis* in the"Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine.

The ornamental value of individual plants or groups in plantings was assessed using the scale developed by V.M. Ostapko and N.Yu. Kunets (Ostapko & Kunec, 2009). The developed scale includes 20 main characteristics that characterize the overall appearance, decorative qualities of flowers, inflorescences, shoots, leaves, fruits, and the individual as a whole.

Each sign of decorative qualities is evaluated from 1 to 5 points. To determine the significance of each feature, transfer coefficients were applied. The total amplitude in the overall assessment of the decorative qualities of the plant is calculated by multiplying the decorative qualities estimates by the transfer coefficient. Since the flowers in the inflorescences of representatives of the family *Araceae* are small and inconspicuous, then instead of the decorative assessment of a single flower, we suggest applying similar criteria to assess the decorative qualities of the spathe. Signs of decorative qualities have the following transfer coefficients: the characteristics of the general appearance of the plant: decoration period - 1; duration of flowering - 3; the nature of flowering - 2; Shoot: peduncle strength - 2; color - 1; Leaf: leaf formations - 1; color - 3; resistance to burning - 2; durability - 1; Inflorescence: number on the generative shoot - 2; number of simultaneously open flowers in an inflorescence - 3: density - 2; size - 1; Fruit: color - 3; resistance to fruit shedding - 2; Spathe: the number of open spathe on the plant at the same time - 3; spathe diameter - 1; color - 3; resistance to burning - 2; lodging resistance - 2.

RESULTS AND DISCUSSIONS

In the "Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine, *L. camtschatcensis* is cultivated in the research and introduction area named after V.V.

Mitin (quarter 3). It is planted in an artificial mini-pond (2.5×1.2 m) with a depth of 0.4 m (Figure 1). In the water body, 10 plants of *L. camtschatcensis* were planted, with heights ranging from 10-15 cm and roots measuring 15-20 cm in length, spaced 20-30 cm apart and positioned 5-10 cm deep from the water surface, along the northern side of the pond, under conditions of almost full illumination (Figure 2).

Figure 2. Group of *L. camtschatcensis* plants one year after planting in the artificial mini-pond at the research and introduction area named after V.V. Mitin

During the years 2013-2022, *L. camtschatcensis* has proven itself in relation to ecological factors as a hydrophyte, microthermophyte, sciophyte, mesotroph, glycophyte, and neutrophyte. Over the years of research, in the artificial mini-pond alongside *L. camtschatcensis*, communities have formed with *Butomus umbellatus* L.*, Carex strigosa* Huds., *Equisetum hyemale* L.*, Mimulus luteus* L., *Nymphoides peltata* (S. G. Gmel.) O. Kuntze, *Potentila indica* (Andrews) T.Wolf, *Schoenoplectus lacustris* (L.) Palla, and *Schoenoplectiella mucronata* (L.) J. Jung & H.K. Choi (Figures 3-4).

Figure 3. Community of hydrophytes, including *L. camtschatcensis*, in the culture of the "Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine during the period of mass flowering

Figure 4. Community of hydrophytes, including *L. camtschatcensis*, in the culture of the "Sofiyivka" National Dendrological Park of the National Academy of Sciences of Ukraine at the end of the flowering period

Every year (up to the age of 10), the height of the plants increased due to the growth of leaves. At the age of 4-5 years, the formation of one inflorescence per plant was noted, and the length of the leaves with petioles was more than 40 cm. The following year, some individuals formed two inflorescences. (Figure 5). The number of seeds in the fruit (72±54 pcs.) depended on the size of the fruits, which were 66.0±14.0 mm in length and 22.0±9.0 mm in width (Chikov, 2020). The introduced plants of *L. camtschatcensis* produce high-quality seeds, with a seed germination of about 50%, indicating the success of their introduction in the conditions of the Right-Bank Forest-Steppe of Ukraine.

Figure. 5. *L. camtschatcensis* during fruiting in the culture of "Sofiyivka" National Dendrological Park

The life cycle of *L. camtschatcensis* begins in early spring (mid April to early May) with flowering, which lasts for about a month. Typically, a generative shoot covered in white bracts appears first, with two leaves on the sides containing underdeveloped leaf blades of lanceolate shape, serving a protective function. Heterophylly is characteristic of *L.*

camtschatcensis. Two weeks later, these leaves bend towards the water and gradually wither, while true leaves emerge from the center near the inflorescence, growing almost vertically. After flowering, the leaves unfold sequentially and grow very rapidly. In June or early July, the number of green leaves reaches its maximum, the largest leaf appears, and the plant biomass is at its peak. From mid-August, leaves with underdeveloped leaf blades develop, and in September, a conical winter bud appears, covered with a small stiff leaf with an underdeveloped leaf blade. From the end of June to mid-August, the seeds float and ripen. The gel-like membrane gradually decomposes and disintegrates. The scattered seeds germinate during the year or after winter. The seedling continues to grow for several years to become a generative plant. Flowering occurs in individuals with leaf lengths ranging from 30 to 70 cm, the size of which depends on the illumination of the area (Fujita, 1997). *L. camtschatcensis* also reproduces vegetatively. In this case, a lateral bud sprouts from the rhizome, which can develop into a vegetative plant. Although *L. camtschatcensis* has two reproductive systems, asexual reproduction is rare (Hokkaido Development Association, 1996).

The visual assessment of the decorative qualities of *L. camtschatcensis* in the collection of hydrophilic plants at the Sofiyivka dendrological park was conducted using the scale developed by V.M. Ostapko and N.Yu. Kunets (Ostapko & Kunec, 2009), which includes 20 main characteristics describing the overall appearance, decorative qualities of the flower, inflorescence, shoots, leaves, fruit, and the plant as a whole (Table 1).

Table 1. Assessment of the decorative qualities of *L. camtschatcensis* according to the scale developed by V.M. Ostapko and N.Yu. Kunets (2009)

Characteristic	Scoring range (min-max)	Transfer coefficient	Feature assessment	Explanation
Characteristics of the general appearance of the plant:				
Decorative Period	2-5	1	3	A specific period of the growing season (spring, summer (April - June))
Duration of flowering	3-15	3	15	Flowering duration exceeds 30 days
Nature of flowering	6-10	2	6	Once per season
Stem:				
Strength of the flower stalk	2-10	2	10	The flower stalks are thick, resistant to bending, and only bend towards the water when the fruits ripen.
Color	1-5	3	3	Light green color, somewhat contrasting with the color of the leaves.
Leaf:				
leaf shape	1-5	1	3	The basal leaves are modified, with the first ones to wither being. The largest the middle ones.
color	3-15	3	6	Light green color
resistance to fading	2-10	2	4	The color of the leaves fades, reducing the decorative effect.
longevity	3-5	1	3	The highest decorative value is observed from the end of flowering to the ripening of the fruits.
Inflorescence: number of flowers on the generative shoot	2-10	2	2	From one to four inflorescences on a generative shoot.
number of flowers simultaneously open in the inflorescence	3-15	3	12	50% of the total amount.
density	6-10	2	10	A type with dense inflorescences.
size	1-5	1	5	Inflorescence length is 15 cm or more.

Bractal leaf spathe: Number of simultaneously open spathe per plant	9-15	3	9	Less than 50%.
spathe diameter (parcel)	1-5	1	5	The diameter of the flower is more than 2 cm.
color	3-15	3	6	Light color.
resistance to fading	2-10	2	4	Fades and changes shade.
lodging resistance	4-10	2	10	Flowers are up to 20% shedding
Fruit: color	3-15	3	15	Fruits are prominently visible, causing admiration
resistance to fruit shedding	4-10	2	10	Shedding occurs only during ripening.
Total score	**40-200**		**141**	**High decorative value.**

In the Lviv Botanical Garden, *L. camtschatcensis* grows at the head of the reservoir with a muddy substrate and shade covering about 80% of its area, showing signs of eutrophication due to the lack of water drainage – the sluice gate is blocked, and there is a significant amount of leaf litter (Shrubovych, 2006). *L. camtschatcensis* in the reservoir is represented by robust plants with large (up to 90×27 cm) leaves and inflorescences with white spathes (approximately 30 cm long). The species thrives well in association with *Caltha palustris* L., *Carex strigosa*, *Filipendula ulmaria* (L.) Maxim., *Geranium phaeum* L., *Houttuynia cordata* Thunb., *Houttuynia cordata* 'Chameleon', *Iris pseudacorus* L., *Lysichiton americanus*, *Lysimachia nummularia* L., *Lysimachia vulgaris* L., *Matteuccia struthiopteris* (L.) Tod., *Plantago major* L., *Ranunculus repens* L., *Thelypteris palustris* Schott., under the canopy of alders (*Alnus* Mill.), willows (*Salix* L.), and poplars (*Populus* L.). Analysis of the experience of cultivating *L. camtschatcensis* in the "Sofiyivka" Dendropark in open areas and in shade in the Lviv National University Botanical Garden showed that the species has higher decorative qualities of habitus in shaded conditions. In our collection, situated in an open sunny area, we observed leaf scorching, so the plants require additional shading.

CONCLUSIONS

In cultivation conditions, *L. camtschatcensis* demonstrated high decorative qualities in terms of: duration of flowering, robustness of the flower stem, density and size of inflorescences, size of the spathe leaf, coloration, and dropping of fruits. The total score of 141 points (range 40-200) confirms the high decorative value of *L. camtschatcensis* in cultivation. In terms of ecological factors, the studied species has proven itself as a hydrophyte, microthermophyte, sciophyte, mesotroph, glycophyte, and neutroph.

In the conditions of the Right-Bank Forest-Steppe of Ukraine, *L. camtschatcensis* is resilient in artificial groupings with *Butomus umbellatus* L., *Caltha palustris* L., *Carex strigosa*, *Equisetum hyemale* L., *Filipendula ulmaria*, *Geranium phaeum*, *Houttuynia cordata*, *Houttuynia cordata* 'Chameleon', *Iris pseudacorus*, *L. americanus*, *Lysimachia nummularia*, *Lysimachia vulgaris*, *Matteuccia struthiopteris*, *Mimulus luteus*, *Nymphoides peltata*, *Plantago major*, *Potentila indica*, *Ranunculus repens*, *Schoenoplectus lacustris,* and *Schoenoplectiella mucronata*, *Thelypteris palustris*.

Under certain conditions, this species can be successfully used for landscaping decorative water bodies in the conditions of the Right-Bank Forest-Steppe of Ukraine. However, considering that another species, *Lysichiton americanus* Hultén & H.St. John, is recognized as invasive and potentially hazardous in the European Union countries, caution is advised with *L. camtschatcensis*, especially in ecologically sensitive natural zones. It is recommended for use in enclosed water bodies where control measures can be implemented.

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STUDIES REGARDING THE BEHAVIOUR OF 'MONEYMAKER' TOMATOES DURING THEIR MARKETING IN FRESH STATE

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Abstract

The consumption of fresh tomatoes, both in Romania and worldwide, has an important place as far as the vegetable category is concerned. This paper presents the results of the studies carried out for the technological and economic characterization of the 'Moneymaker' tomato in the marketing process in fresh state. The duration of maintaining the quality depends on the ripening stage at harvesting and on the temperature level during storage. The limit period for fresh fruits marketing is 7-9 days at a temperature of 23-24 ^{0}C and of 18-22 days at a temperature of 7-10^{0}C. It was also ascertained that over 97% of the output of the 'Moneymaker' tomato corresponds to the specific quality standard. The fruits harvested in greenhouse or solarium have a homogenous structure by quality categories, the value of quality category coefficient (Q) having values between 2.64 and 2.60.

Key words: *postharvest, quality preservation, storage period.*

INTRODUCTION

Tomatoes are among the most consumed vegetables worldwide, and variety is one of the main factors that define the quality of production and the destination of its valorization (Chira & Chira, 2022; Nirupama et al., 2020).
The spread of this species is due to its adaptability to different cropping systems and different processing conditions. Tomato fruits have a great diversity and superior quality characteristics that meet consumer requirements (Dobrin et al., 2019; Ilie & Mihalache, 2022; Hatami et al., 2013).
Given that the 'Moneymaker' ensured the possibility of delivering uniform batches of high quality tomatoes for most of the year, it was considered necessary to carry out research for technological and economic characterization in the conditions of the Vidra vegetable basin in Ilfov County.
In the literature, the 'Moneymaker' is characterized more from an agro-productive point of view. For the technological characterization of tomatoes, the assessment criteria and factors influencing fruit quality during storage are mentioned in numerous papers (Winsor, 1979: Thole et al., 2021).
Particular attention is paid to the scientific substantiation of the changes that occur in the fruit marking process: colour, flesh firmness and chemical components that determine the nutritional value of the fruit, as mentioned in many scientific studies (Brashlyanova et al., 2014; Kabir et al., 2020; Al-Dairi et al., 2021; Pinela et al., 2022; Thole et al., 2020).
The aim of the present research was to investigate 'Moneymaker' tomato storage period and some quality changes related to the culture systems, as well as the preservation conditions.

MATERIALS AND METHODS

The biological material has been represented by 'Moneymaker' tomatoes obtained from different growing systems (greenhouse and solarium) from a private farm located in the Vidra vegetable basin, Ilfov County (Figure 1). A specific cultivation technology was applied in greenhouse (cycle 1) and the solarium (cycle 1 and cycle 2), respectively (Table 1).

Table 1. Tomato growing conditions, harvesting period and storage conditions

Harvesting period	**Growing system**	**Environmental room**		**Refrigerated room**	
		Temperature (T)(⁰C)	**Relative Humidity (RH) (%)**	**T (⁰C)**	**RH (%)**
1.02. – 22.02	Greenhouse Cycle 1	23-24	65-70	7-8	95-100
27. 07 - 13.08	Solarium Cycle 1	23-24	70-85	9-10	90-95
28.09 – 11.10 First harvest	Solarium Cycle 2 partial protected	20-21	80-85	8-9	90-95
23.10 – 18.11 Second harvest	Solarium Cycle 2 partial protected	19-20	80-85	7-8	90-95

It should be noted that in solarium culture, cycle 2, the total protection of the crop with polyethylene film was not ensured.

Figure 1.'Moneymaker' tomato (own source)

At the harvesting time and at the end of the storage period the following parameters have been determined: the main physical-chemical, such as water content (%), dry total matter (%), dry soluble matter (%), firmness (kgf/cm^2), total titratable acidity (%) and the content of ascorbic acid (mg/100 g fresh weight).
The water content and total dry matter have been determined by using a ventilation oven, at 105⁰C. The content of soluble dry matter was measured by using the Atago electronic refractometer (Figure 2).
The total titratable acidity was measured by titration with a sodium hydroxide (NaOH) 0.1N solution. The content or ascorbic acid was measured using the iodometric method. The pulp firmness was determined by using the hand-held Effegi penetrometer, having an 11mm piston in diameter (Figure 3).

Figure 2. Determination of soluble dry matter, using the Atago refractometer (own source)

Also, the shape index has been calculated (height/medium diameter ratio).

Fig. 3. Determination of fruit firmness, using the hand-held Effegi penetrometer (own source)

The temperature level and the air relative humidity in the storage environment have been measured using the Hanhart thermo hygrometer.
It is known that tomatoes can be harvested at different ripening stages, depending on the destination of the crop, as follows: F0 - green ripening, F1 - when 10-30% of the skin is pinkish-yellow, F2 - when 30-50% of the skin

is pinkish-yellow, F3 - when 50-90% is red, F4 - when 100% is red and F5 - when the fruit contains at least 4.5% soluble dry matter (technological maturity). In the present work there were carried out two harvesting phases: F1 and F3.
The tomatoes fruits were then stored under different environmental conditions, as we can see in (Table 1).
There were used 3 replicates, each represented by 3kg of tomatoes, which were stored in the environmental and refrigerated room, respectively, in the Technology Laboratory of the Faculty of Horticulture Bucharest (Figure 4 and Figure 5).

Fig. 4. Tomato fruits stored in the cooling room (own source)

Fig. 5. Tomato fruits stored in the enviromental conditions (own source)

The economic efficiency of tomato valorization was determined by establishing the structure of production by quality category (Q) using the formula:
Q = Kq/100, where K = 3 for extra quality; 2 for quality 1; 1 for quality 2 and 0 for those intended for industrialisation, and q is the percentage of the quality category.

RESULTS AND DISCUSSIONS

1. The quality of tomato fruit at harvest

The results of the physical-chemical analysis carried out on tomato fruits are presented in Tables 2 and 3. It can be seen as a good homogenity in the case of this cultivar.

Table 2. The main physical characteristics of tomato fruits at harvest time

Growing system	Maturation phase	Shape index	Caliber (40-47 mm)		Caliber (47-57mm)	
			Diameter (mm)	Weight (g)	Diameter (mm)	Weight (g)
Greenhouse Cycle 1	F1 F3	1.02 1.02	45 45	41 41	57 57	74 74
Solarium Cycle 1	F1 F3	1.03 1.03	43 43	40 40	52 52	75 75
Solarium Cycle 2 first harvest	F2	1.10	47	52	56	81
Solarium Cycle 2 second harvest	F1	1.08	46	50	48	69

Table 3. The main chemical characteristics of tomato fruits at harvest time

Growing system	Water (%)	Total dry matter (%)	Soluble dry matter (%)	Ascorbic acid (mg/100g FW)	Total titratable acidity (%)
Greenhouse Cycle 1	94.07 94.07	5.93 5.93	5.4 5.6	17.03 24.33	0.43 0.45
Solarium Cycle 1	94.48 94.75	5.52 5.25	5.3 5.1	30.79 30.79	0.37 0.36
Solarium Cycle 2 first harvest	94.71	5.29	5.1	35.95	0.40
Solarium Cycle 2 second harvest	93.26	6.74	5.4	39.75	0.30

Thus, in terms of the shape index, the values determined were close to 1, which meets the requirements for the spherical shape, a characteristic feature of this cultivar.

The size of the tomatoes at harvest was average, with diameter values between 43 mm and 57 mm and a weight of 40-81 g.

The water content of tomatoes ranged from 93.26% to 94.75%, while total dry matter ranged from 5.25% to 6.74%. The soluble dry matter content ranged from 5.1 to 5.6%.

The ascorbic acid content ranged from 17.03 mg/100 g FW to 39.75 mg/100 g FW and the total titratable acidity (as malic acid) ranged from 0.3% to 0.45%. The results obtained fall within the specific values defining good quality tomato fruit (Dobrin et al., 2019; Abiso et al., 2015).

2. Duration of tomato quality maintenance after harvesting

The results obtained, presented in Table 4, confirm that the duration of the interval from harvesting to reaching the stages of eating maturity (F4/F5) is directly influenced by the degree of ripeness of the tomatoes at harvest.

Harvested from greenhouses or solariums at the F1 stage, tomatoes reach consumption maturity after 5 days at 23-24^{0}C and after 14-15 days at temperatures ranging from 7 to 10^{0}C.

In the case of tomatoes harvested at F3 stage, the fruit reached consumption maturity in 2 days at 23-24^{0}C and 5-7 days at 7-10^{0}C. After reaching consumption maturity (F4), the tomatoes continue to maintain their specific quality for fresh processing for a period that depends mainly on the temperature values during storage. If we consider the time interval from harvesting to the possible limit for fresh processing, the influence of the ripening stage at harvest is not so evident.

Thus, tomatoes harvested in the F1 phase, which have reached the maturity for consumption in 5 days at 23-24^{0}C, can still be used in good conditions after another 3-4 days, so the total period is 8-9 days.

If harvested at the F3 stage, tomatoes reach eating maturity in 2 days, but they can also be harvested in good condition after another 5-6 days, the total period in this case being 7-8 days. Basically, the two periods with tomatoes harvested in F1 and F3 phases are almost equal until the consumption limit is reached.

At temperatures of 7-10^{0}C, the total period from harvest to consumption was extended to 20-22 days.

In cycle 2, tomatoes harvested at F1 stage and kept at 20-21^{0}C were harvested after 6 days and those kept at 8-9^{0}C after 13 days. Tomatoes obtained at the 2nd harvest were harvested after 8 and 14 days respectively.

Table 4. The storage period of tomato fruits, depending on temperature levels and harvesting maturation phase

Growing system	Maturation phase at harvest	Temperature level during storage (^{0}C)	The period from harvest until full maturation F4/F5 (days)
Greenhouse	F1	23-24 7-8	5 F4 8 F5 14 F4 22 F5
	F3	23-24 7-8	2F4 7F5 5F4 20F5
Solarium Cycle 1	F1	23-24 9-10	5F4 9 F5 15F4 20F5
	F3	23-24 9-10	2F4 8F5 7F4 18F5
Solarium Cycle 2 (first harvest)	F1	20-21 8-9	6F4 10F5 13F4 17F5
Solarium Cycle 2 (second harvest)	F1	19-20 7-8	8F4 12F5 14F4 19F5

3. Changes seen in ripening tomato fruit after harvesting

Tomatoes are climacteric fruits, that continue their ripening process after harvesting, with changes in their physical-chemical characteristics. Thus, at ripening, flesh firmness had values between 4 and 7.6 kgf/cm^2 (Table 5). After tomatoes storage in different temperature conditions, due to solubilization of pectic substances, firmness decreased by 19-47% at 23-24^{0}C reaching values of 2.8-4 kgf/cm^2. The most relevant reduction in firmness was recorded in tomatoes harvested in stage 2 of the solarium - cycle 2 (from 7.6 to 3 kgf/cm^2) in 26 days at 7-8^{0}C.

This was followed by greenhouse-grown tomatoes harvested at F1 stage and kept at 8-10^{0}C for 22 days (from 6.7 to 3.38 kgf/cm^2), and then by those in the glasshouse, cycle 2, harvested at the same stage (from 6.5 to 3.4 kgf/cm^2).

It was observed that irrespective of the type of crop, tomatoes harvested in the F1 phase and stored at 8-10^{0}C for more than 20 days lose firmness to a greater extent than those harvested in the F3 phase, even when the storage period was the same.

The weight losses determined during storage were higher in tomatoes harvested at F1 stage (from 4.7 to 5.7%) at approximately the same temperature (8-10^{0}C) and storage time (20-22 days). The same influence of ripening phase was found at 23-24^{0}C (4.1% to 4.7% at F1 phase and 2.9-3.2% at F2 phase) (Table 6).

The daily weight losses were reduced as the shelf life was extended (Thole et al., 2020).

Table 5. The evolution of tomato fruit firmness depending on the harvesting maturation phase and temperature level during storage

Growing system	Maturation phase at harvest	Firmness at harvest time (kgf/cm^2)	Temperature (^{0}C)	Storage period (days)	Firmness at the end of storage (kgf/cm^2)	Firmness loss (%)
Greenhouse	F1 F1	6.7 6.7	7-8 23-24	22 8	3.38 4.00	50.7 40.0
	F3	4.6 4.6	7-8 23-24	20 5	14.17 3.73	10.8 19.0
Solarium Cycle 1	F1	6.5 6.5	9-10 24-24	20 9	4.20 3.40	35.0 47.0
	F3	4.0 4.0	9-10 23-24	18 8	3.20 2.80	25.0 30.0
Solarium Cycle 2 (first harvest)	F1	7.6 7.6	8-9 20-21	13 6	5.7 4.8	25.0 36.0
Solarium Cycle 2 (second harvest)	F1	7.3 7.3	7-8 19-20	14 8	3.0 3.4	58.0 53.0

Table 6. The weight losses of tomato fruits during storage period

Growing system	Maturation phase/ Temperature (^{0}C)	Weight losses (%)				Storage period (days)
		after 24 h	after 48 h	after 72 h	End of storage	
Greenhouse	F1/7-8	0.4	0.7	1	4.5	22
	F2/7-8	0.2	0.4	0.5	2.5	20
	F1/23-24	0.7	1.0	0.6	4.1	8
	F3/23-24	0.2	0.8	1.2	2.9	5
Solarium Cycle 1	F1/9-10	0.6	0.9	1.0	5.7	20
	F3/9-10	0.4	0.6	0.9	4.3	18
	F1/23-24	0.6	1.1	1.5	4.7	9
	F3/23-24	0.3	0.9	1.6	3.2	8
Solarium Cycle 2 (first harvest)	F1/8-9	0.2	0.4	0.7	2.9	13
	F1/20-21	0.7	1.0	1.8	6.2	6
Solarium Cycle 2 (second harvest)	F1/7-8	0.3	0.5	0.7	4.1	14
	F1/19-20	0.2	0.6	1.4	5.4	8

Regarding the results obtained on the evolution of the main physical-chemical characteristics that define the quality of tomatoes, from the data presented in Table 7, during ripening, after harvesting there were specific morphological, physical and biochemical changes. Thus, during storage there was a slight increase in the total dry matter content, due to water loss through transpiration and concentration of the cell juice.

Tomatoes stored at 23-24^{0}C showed a slight decrease in total titratable acidity, due to more intense oxidation processes at higher temperatures, compared to 7-8^{0}C, where a slight decrease was determined.

Ascorbic acid content increased more during storage mainly in greenhouse tomatoes harvested at F1 phase and stored at 23-24^{0}C (29.19 mg/100g compared to 17.03 mg/100 g at harvest) according to the previous published results (Al-Dairi et al., 2021).

4. The economic efficiency of the valorization of 'Moneymaker 'tomatoes

The quality structure of the production of the 'Moneymaker' tomato grown in the two cropping systems is shown in Table 8.

It results that in all cropping systems, out of the total production, 97.9-99.2% falls within the requirements of the quality standard for fresh tomatoes.

The coefficient of the quality categories, which characterizes the homogeneity of the cultivar in terms of quality, in the three cropping systems, varied between 2.46 and 2.60 (Table 8). The close values of this coefficient indicate the homogeneity of the quality of the cultivar in all cropping systems.

Table 7. The main physical-chemical characteristics of tomato fruits at the end of the storage period

Growing system	Maturation phase at harvest	Storage temperature (^{0}C)	Storage period -day)	Water (%)	Total dry matter (%)	Soluble dry matter (%)	Ascorbic acid (mg/100g)	Total titratable acidity (%)
Greenhouse	F1	23-24	8	92.85	7.15	6.4	29.19	0.41
	F1	7-8	22	93.87	6.13	6.0	25.26	0.46
	F3	23-24	5	94.05	5.95	5.7	20.27	0.34
	F3	7-8	20	93.90	6.09	5.6	20.53	0.43
Solarium	F1	23-24	9	93.95	6.05	5.8	39.67	0.34
	F1	9-10	20	93.96	6.04	5.8	28.96	0.39
	F3	23-24	8	94.3	5.7	5.5	27.21	0.35
	F3	9-10	18	93.88	6.12	5.9	30.29	0.34

Table 8. The quality structure of tomato fruits in the valorisation process

Growing system	**Selling period**	**Quality structure (%)**				**Total according quality standard**	**The coefficient of the quality category**
		Extra	**First quality class**	**Second quality class**	**Industry class**		
Greenhouse Cycle 1	20.03-1.07	72.0	12.0	15.2	0.8	99.2	2.55
Greenhouse Cycle 2	25.09-31.12	76.0	9.7	13.4	0.9	99.1	2.60
Solarium	10.06-30.09	67.2	13.8	16.9	2.1	97.9	2.46

CONCLUSIONS

Maintaining the quality of the tomatoes after harvesting until they are ready for consumption can be ensured for:

- 5 days at 23-24^{0}C and RH 70-85% for those harvested at F1 phase;
- 2 days at 23-24^{0}C and RH 70-85% for those harvested at F3 phase;
- 14-15 days at 7-10^{0}C and RH 90-95% for those harvested in F1phase;
- 5 days at 7-10^{0}C and RH 90-95% for those harvested in F3 phase.

Once tomatoes have reached the maturity for consumption, they can be used for a longer period, reaching a total of :

- 7-9 days at 23-24^{0}C and RH 70-85%;
- 18-22 days at 7-10^{0}C and RH 90-95%.

Changes in the physical-chemical characteristics during storage do not adversely affect the possibility of consuming them fresh within the specified shelf-life.

The daily weight loss decreased as the shelf life was extended.

Economic quality indicators of the 'Moneymaker' tomato show that:

- 97.9-99.2% of the total production meets the quality standard for fresh tomatoes;
- the coefficient of the quality categories (Q) had values between 2.46 and 2.60, indicating the homogeneity of the quality structure in the three cropping systems.

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ASSESSMENT OF NATURAL REGENERATION IN CROPS FROM THE APUSENI MOUNTAINS

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Abstract

The choice of the areas for analysis was made according to a series of relevant principles such as the presence of the seedbed and its nature, the age of the stand to be above the lower limit of 50 years, the consistency of the stand below the value of 0.7. From the analyzes carried out, it was possible to identify the main forest types of influence of major seasonal factors (climatic, geomorphological, pedological) on the establishment and evolution of natural regeneration. It was considered, the synthesis of the way in which these factors imposed a certain distribution of the seed and the importance of this aspect for the structure of the arboretum.
From the point of view of the influence of seasonal factors on the settlement, the conducted study indicates good regeneration conditions for the majority resort type 2322 (spruce mountain) correlated with a location on slopes with slight or moderate slopes, sunny exposures at high altitudes and soils with volume middle edaphic.

Key words*: functional group; resort type; forest type; geomorphological form; litter type.*

INTRODUCTION

The forest is a community of plants and animals that live in interdependence and has an essential role in people's lives, providing products such as wood, food, oils, resin, but also creating environmental conditions for different ecosystems (Pecinginǎ, 2019). Thus, the forest provides raw materials necessary for the development of numerous industrial branches in both developed and less developed states (Cseke et al., 2006; Ferguson, 1996; Hassan et al., 2005; Kozlowski, 2002; Negulescu et al., 1973; Vlad et al., 1997). However, the forest is subject to numerous biotic and abiotic risks (Achard et al., 2006; Bryant et al., 1997; Kozlowski, 2000). Providing numerous benefits for the environment and population, ensuring forest restoration is becoming increasingly important. Thus the sustainability of forests is achieved through adequate and timely regeneration of forests (Chazdon & Guariguata, 2016; Dey, 2014; Dey et al., 2019; Schelhas et al., 2021).
Natural regeneration is defined as the renewal of an arboretum by seeding or sprouting and is a slow and unpredictable process determined by the interaction of seeds with site factors (Duncan, 1954). Very important in high-altitude forests is the pattern of regeneration through spatial and temporal dynamics (Carrer et al., 2013; Spracklen et al., 2013). Regeneration can occur in 3 cases: where arboretum density has not been altered for a long time, where areas have been threatened by biotic or abiotic stress, and following regeneration cuts (Pardos et al., 2005; Serrada, 2003). As for the natural regeneration, it is different depending on the species, so in the case of the white spruce the best conditions are with 4-5 old seedling spruces per hectare in the cut areas or dense crops on the edges of the cuts, and the most favorable soil is the mineral one (Gärtner et al., 2011). In the forests of the northwest of the Transylvanian Plain, a natural regeneration of around 70% was achieved (Holonec et al., 2008). Natural regeneration is different depending on abiotic and biotic factors. Differences in natural regeneration have been found in the tropics depending on rainfall and environmental factors (Khaine et al., 2018).

Historical events have affected the genetic structure of forest plants and especially their distribution. Thus, in addition to forest species, other plant species were also affected, especially in the mountainous area (Höhn, 1998; Tóth et al., 2017). An area of Europe considered with diversity is the Mediterranean area, the southern peninsulas, including the Balkans, which have preserved genera and species of trees from ancient times, being considered the main area of post-glacial recolonization (Cowling et al., 2015; Gömöry et al., 2020). The diversity of forest species is influenced by many factors such as the geological substratum (for example species diversity in the karst area (Csergo et al., 2014), soil (Miles, 1985), altitude, climate change, etc. (Cowling et al., 2015; Liang & Wei, 2020; Pauli et al., 2012; Casiana Mihuț & Niță L., 2018). Climatic changes implying an increase in temperature, a change in precipitation, a decrease in the thickness of the snow cover cause a change in the types of vegetation and even a change in the methods of forest regeneration (Díaz et al., 2021; Gaál et al., 2012; Hajnalka et al., 2018; Hufnagel, 2017; Kiedrzyńska et al., 2021; Sipkay et al., 2010).

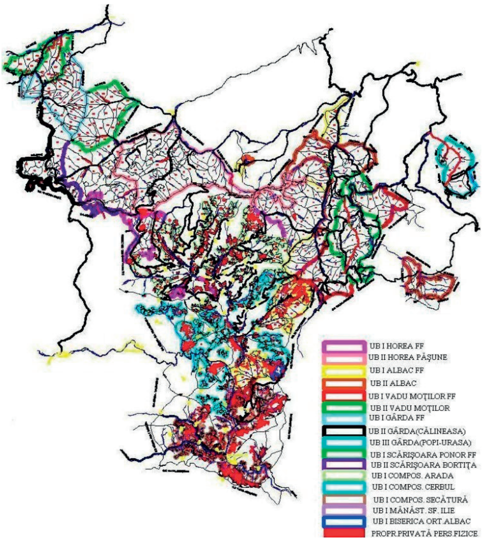

Figure 1. The map of Horea Forest District, Apuseni (scale 1:20000)

Very important in the conditions of climate change is the urgent implementation of policies for sustainable development, as well as measures to support biological diversity (Ferris & Humpherey, 1999; Lindenmayer et al., 2000; Mihuț Casiana et al., 2022; Zalewski, 2015; Zanchi et al., 2007).

Study area. The Horea Forest District in the Apuseni Mountains is located in the Biharia-Muntele Mare-Muntele Vlădeasa massifs, in the basins with affluent valleys of Ariesul Mare, the middle part and the upper parts of Someșul Rece and Someșul Cald. Administratively, the forest district carries out its activity within the counties: Alba - 4233 ha, Bihor - 180 ha and Cluj - 11374 ha, having a total area of 15787 ha (Figure 1).

MATERIALS AND METHODS

In order to be able to analyze the role that seasonal factors play in the evolution of natural regeneration, we considered the P.U. I Scărișoara-Ponor, from the Horea Forest District.

The selection of surfaces for analysis was made according to a series of relevant principles, namely: the presence of usable seedlings regardless of its age and nature, the age of the arboretum must fall above the lower limit of 50 years, an arboretum consistency below 0.7. In addition, only those surfaces were selected which, based on the works carried out, were affected in the recent past by disturbing elements such as: wind blows, snow, pest attacks, drying, thus decisively influencing the evolution of the settlement.

Of the total surface of P.U. I Scărișoara-Ponor which is 1553.3 ha (52 parcels and 216 subparcels), a total of 187.9 ha (2 parcels and 17 subparcels) fit the criteria, but due to the fact that the seedlings are located only on certain plots in the administrative units, the effective area occupied with seedlings and subject to analysis is 56.5 ha, about 3.6% of the total area of P.U. I Scărișoara-Ponor.

The quantification and analysis of the data was done in relation to the most important seasonal factors that can influence the development of natural regeneration, namely: functional group; type of resort; type of forest; soil type; geomorphological form; the exposure; the litter; the age of the seedling and the treatments carried out (applied). The two directions followed are represented by the distribution of the area with seedlings in relation to the stational factor as well as the distribution of the seedlings per each seasonal factor.

RESULTS AND DISCUSSIONS

Analyzing the distribution of the naturally regenerated area from P.U. I Scărișoara-Ponor (Figure 2), we may observe the presence of only one type of functional group, namely 1-5 L (forests from national parks). This aspect is normal because the functional group in question occupies the largest area at the level of the studied production unit, which is actually included in the Apuseni Natural Park.

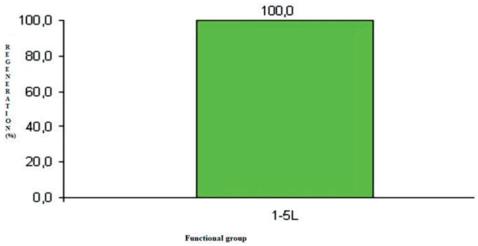

Figure 2. The distribution of the naturally regenerated surface in relation to the functional group from P.U. I Scărișoara-Ponor

Regarding the distribution of the seedlings by functional groups, we distinguish a certain balance in the case of P.U. I Scărișoara-Ponor between the mixed type (50.6%) and the intimate one (41.9%). A relatively low percentage is recorded in the case of the distribution type in groups, of 7.4% (Figure 3).

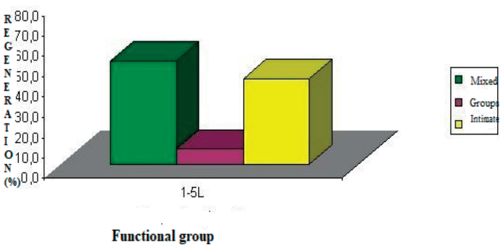

Figure 3. The distribution of seedlings by functional groups in P.U. I Scărișoara-Ponor

The general perspective of the P.U. I Scărișoara-Ponor indicates a balance between the combined spread of the seedlings and the uniform spread over the entire surface of a single functional group.

Following the analysis of the distribution of the naturally regenerated surface in relation to the type of station in P.U. I Scărișoara-Ponor, we may observe that most of the natural regeneration has developed within the station type 2322 (spruce-type montanous Bm), namely 57.5%, and within the station type 3332 (mix-type montanous Bm), of 28.2% (Figure 4).

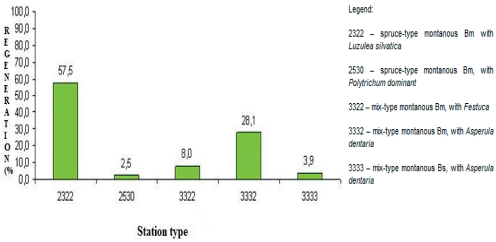

Figure 4. The distribution of the naturally regenerated surface in relation to the station type in P.U. I Scărișoara-Ponor

From the point of view of how the seedlings are distributed by station type, the natural regeneration from P.U. I Scărișoara-Ponor may be remarked due to the predominance of the mixed and intimate mode of distribution (Figure 5). The most important proportions are found on the two types of majority stations: 2322 (mixed 22.7%; intimate 29.2%) and 3332 (mixed 20.2%; intimate 6.2%). The way of distribution into groups of the seedlings presents a lower percentage, having present the station type 2322 (5.7%), respectively 3332 (1.7%).

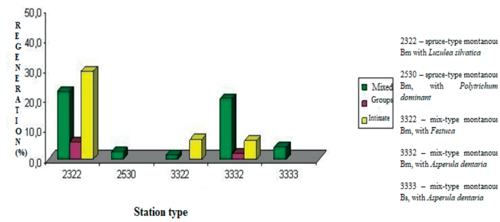

Figure 5. The distribution of seedlingst by station types in P.U. I Scărișoara-Ponor

The natural regeneration in P.U. I Scărișoara-Ponor developed on a high number of station types. Taking into account the fact that only the two stands affected by disturbing elements were taken into account in the analysis, we may support the idea that the high percentages of seedlings are a relevant indicator of the station's exposure to these elements. In this sense, resort types 3322 and 3332 have the highest degree of exposure. The way of distribution of the seedling has a balanced arrangement between the majority mixed and intimate types.

Forest type is another stational factor that plays an important role in the development of natural regeneration. In P.U. I Scărișoara-Ponor, the seedlings grow exponentially in relation to the type of forest 1141 (spruce-type with *Luzula silvatica*), of about 65.5%. A high percentage is also located within the forest type 1341 (mixture of coniferous and beech, on skeletal soils), of about 20.2%. The other types of complementary forest are characterized by a rather weak representation (Figure 6).

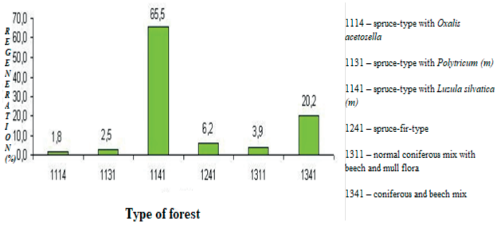

Figure 6. The distribution of the naturally regenerated surface in relation to the type of forest in P.U. I Scărișoara-Ponor

The distribution of seedlings in P.U. I Scărișoara-Ponor per types of forest highlights a high distribution on the intimate grouping mode from F.T. 1141, of 35.8%. The mixed grouping mode is present on the forest type 1141 (24.1%), 1341 (20.2%) and less on the forest type 1311 (normal coniferous mix with beech, with mull flora), of 3.9% (Figure 7).

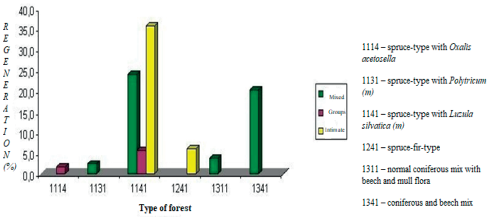

Figure 7. The distribution of the seedling by forest types in P.U. I Scărișoara-Ponor

Based on the analyzed data, we may conclude that the forest type that offers good conditions for the development of natural regeneration is the forest type 1141. In the studied perimeter, the seedlings are located on each type of forest, both individually, through a single way of distribution, and through two, or more isolated through three types, of its organization.

Another seasonal factor that has a major impact on natural regeneration is the type of soil (Figure 8). It greatly influences the seedlings due to the fact that it is a stable component of the forest ecosystem, whose specific characteristics can change the evolution of certain species from one area to another. From the perspective of this aspect, the studied perimeter is characterized by a strong development of natural regeneration on the soil type 4201 (typical podzol), respectively 3301 (typical dystricambosol). The soil type 3306 (gleyed dystricambosol) is very poorly represented at the level of the studied perimeter, the proportion of its representation being approximately 2.5%.

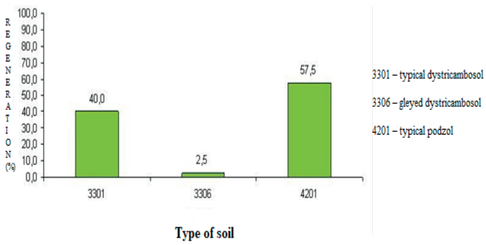

Figure 8. The distribution of the naturally regenerated surface in relation to the type of soil in P.U. I Scărișoara-Ponor

The organization of the seedlings in the studied perimeter is dominated by high percentage values for the intimate distribution mode on the type of soil 4201 (29.2%), respectively mixed within the type of soil 3301 (25.5%) (Figure 9).

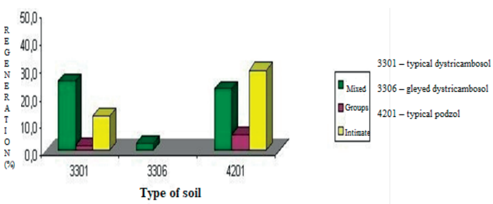

Figure 9. The distribution of seed by soil type in P.U. I Scărișoara-Ponor

The geomorphological shape of the slope (Figure 10) also represents an edifying factor for the evolution of natural regeneration in the perimeter affected by disturbing element. The highest percentages of seedlings in the studied area are located on the middle wavy slopes (53.8%), respectively on the wavy ones (32%). As a particular element, the presence of regeneration can also be noted on the middle flat slopes.

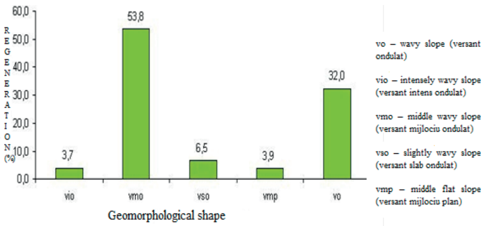

Figure 10. The distribution of the naturally regenerated surface in relation to the geomorphological shape in P.U. I Scărișoara-Ponor

Analyzing the way the seedlings are organized on geomorphological shapes in the studied area, the presence of a generally valid characteristic also found in the case of the other exemplified factors is noted here as well. The area under study is characterized in this sense by the presence, at the level of the dominant and middle wavy slopes, of all forms of seedling organization (intimate, in a proportion of 32%, mixed, 20% and per groups, 1.8%). High percentage values are also recorded in the case of mixed regeneration, on wavy slopes (26.7%) (Figure 11).

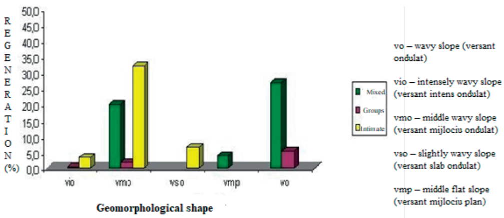

Figure 11. The distribution of the seedlings on geomorphological shapes in P.U. I Scărișoara-Ponor

The exposure of forest surfaces has a major impact on the establishment and evolution of natural regeneration, correlated with the light requirements of forest species. The best growing conditions are recorded on the southern slopes, which are warmer and sunnier, with a long vegetation season. On the other hand, on the northern slopes, the conditions are harsher, here developing species such as fir or beech. The perimeter under study is individualized by a strong regeneration on the southern slopes, of 35.6%. High percentage values are also recorded in the southeastern (17%) and southwestern exposures (14.9%). As for the northern exposures, they occupy 14.0% of the naturally regenerated surface and affected by disturbing elements, while the slopes with a northeast orientation 12.2%, and those on the northwest 5.5% (Figure 12).

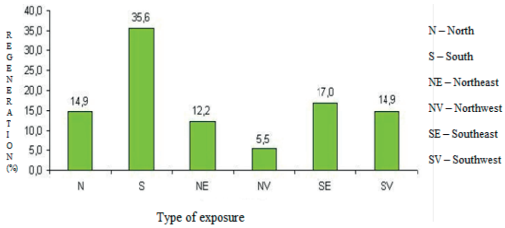

Figure 12. The distribution of the naturally regenerated surface in relation to the type of exposure in P.U. I Scărișoara-Ponor

The studied area is characterized by a balance in terms of the mixed distribution of the seedlings on the types of exposures in which it is present (Figure 13). The highest percentages are recorded on the south-oriented (11.9%) and north-oriented slopes (9.6%). The intimate organization of natural regeneration is well developed on the southern slopes (23.7%), but also on the southwestern ones (14.9%). The last type of organization, per groups, appears sporadically on the northern and northwestern slopes.

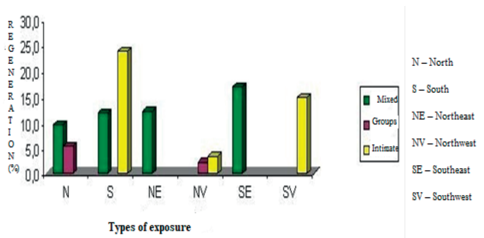

Figure 13. The distribution of seedlings by types of exposure in P.U. I Scărișoara-Ponor

The high percentages for southern, sunny exposures are absolutely normal, it being known that the seedlings develop best in the presence of favorable conditions of light and heat.

The installation of natural regeneration is not possible without an appropriate structure of the vegetation cover at ground level. The seedlings in the studied production unit is structured in an overwhelming majority on a normal-continuous litter type, in proportion of approximately 92.2%. The other type of litter, thin-continuous litter type, is present in a rather low proportion (Figure 14).

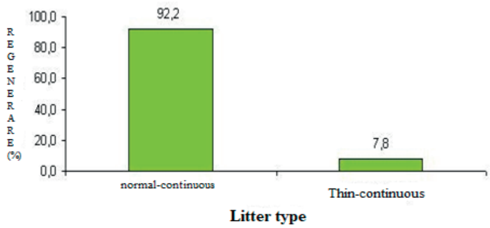

Figure 14. The distribution of the naturally regenerated surface in relation to the litter type in P.U. I Scărișoara-Ponor

The 3 forms of seed distribution fall within the general rule indicated so far. The dominant litter type is indicated by all types of seedling organization (mixed in proportion 45%, by groups 5.3% and intimate 41.9%). The type of complementary litter in this perimeter is individualized by the mixed-group duality.

The age of the seedling is largely dependent on the age of the developed mature arboretum. The disturbing elements that affected the area studied and the treatments carried out also left their mark on the age of the seedlings. The areas studied have a superior development in terms of the seedlings that is around 10 years old (approximately 86.5%). Younger natural regeneration (5 years) is limited in spread (Figure 15).

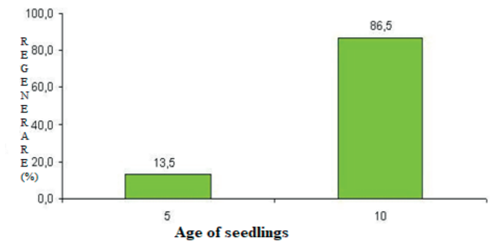

Figure 15. The distribution of the naturally regenerated surface in relation to the age of the seedlings in P.U. I Scărișoara-Ponor

The mixed organization of the seedlings in the studied area (Figure 16) is better distributed among the 10-year age group (40.5%), compared with the seedlings polarized around age 5 (10.1%). The intimate distribution mode is also strongly developed for the 10-year-old seedlings (38.6%) and limited to the 5-year level (10.1%). The weakest form of distribution, that by groups, is only identified in the 10-year-old seedlings (7.4%).

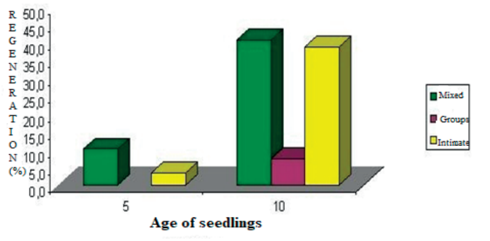

Figure 16. The distribution of regeneration by age categories of the seedlings in P.U. I Scărișoara-Ponor

From the interpretation of the data as a whole, we may conclude that the polarized natural regeneration around the age of 10 years is best represented, in correlation with the combined spread of the seedlings and the uniformity of this at the level of the analyzed surface.

The method of application of the treatment by harvesting exploitable crops has a major impact on the development of the seedlings, both by creating favorable conditions and by protecting them. In the studied perimeter, natural regeneration has developed best on the surfaces with no treatment during the last decade (43.9%), respectively on the surfaces covered with hygiene cuts (35%) (Figure 17).

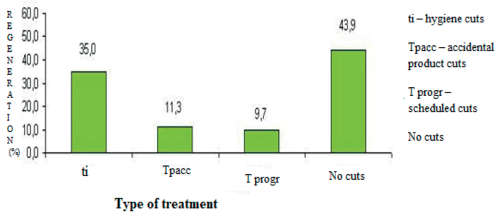

Figure 17. The distribution of the naturally regenerated surface in relation to the type of treatment in P.U. I Scărișoara-Ponor

The surfaces with no treatments in the studied area are characterized by a balance of the three types of seedling organization (mixed 20.7%, per groups 7.1% and intimate 14.9%) (Figure 18).

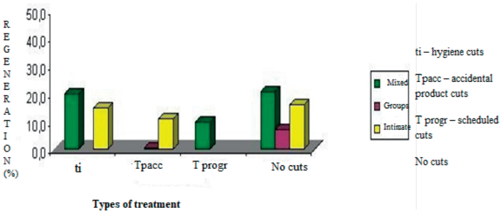

Figure 18. The distribution of the seedlings by types of treatment in P.U. I Scărișoara-Ponor

The overall picture regarding the influence of the treatment on the natural regeneration in the studied area indicates a development of the seedlings in general on the surfaces strongly affected by wind and snow falls (treatment of accidental cuts), in combined forms of spread and those submitted in the last decade to hygiene cuts.

CONCLUSIONS

Due to regional stational peculiarities, the coniferous crops in the Apuseni Mountains are generally characterized by a strong natural regeneration, but the local specificity of each type of station can lead to a series of impediments in terms of the development of the natural seedlings in good conditions.

From the analyzes carried out on the forest areas taken in the study, we had the possibility to identify the main forest types of influence of the major seasonal factors (climatic, geomorphological, pedological) on the establishment and evolution of natural regeneration.

The synthesis of how these factors imposed a certain seedling distribution and the importance of this aspect for the structure of the arboretum was also pursued.

Taking into account the requirements of the main forest species presented (spruce and fir), there are no important limiting factors with negative effects on the development of forest vegetation. On the background of the zonal climate and under the influence of the local relief, we may differentiate characteristic topoclimates, that of a sunny slope and that of a shady slope, which overlap the physiological needs of the species. At the same time, the movement of moist air masses from the southwest and northwest, with intense and frequent frontal activity, leads to a high vulnerability to falls caused by winds.

The higher percentage values of the particularities within the stational and arboreal factors quantified in the present paper are a faithful indicator of the favorability of natural regeneration.

From the point of view of the influence of stational factors on the seedlings, the conducted study indicates good regeneration conditions for the majority type of station 2322 (spruce-type mountanous Bn), correlated with a location on slight or moderate slopes, sunny exposures at high altitudes and deep medium soils with medium edaphic volume.

The studied area is favorable for the natural regeneration of the spruce, being characterized by a medium rating in terms of this species.

The treatments performed in the last decade in the studied area had a minimal influence on the installation of natural regeneration, the seed developing rather as a result of the already existing favorable conditions of heat and light.

Spruce seeding is largely facilitated by the fact that the seed spreads over long distances, but great importance must be given to preventing damage to seedlings from heat and drought, especially on southern slopes, by applying appropriate treatments.

At higher altitudes, a faster release of the seedling from the maternal shelter is indicated, approximately 3-5 years after the seedling is established.

The area under study shows a natural regeneration identifiable only for the spruce, but the creation of stable crops from a silvicultural point of view cannot be obtained without a corresponding natural regeneration for the fir and beech crops, also present in a smaller proportion. Therefore, in order to achieve this objective, the natural regeneration of the fir tree, which takes place under shelter, can be supported by the application of progressive cuts, progressive cuts at the edge of the massif or combinations thereof, with a maximum utilization of the pre-existing seedlings.

In the mixtures of spruce and beech in the studied area, it is necessary to apply progressive treatments, by opening large meshes around the old spruces, in the year of their abundant fruiting, with the aim of favoring the establishment of the spruce and ensuring its advance in growth.

For the regeneration of the beech, the pre-existing seedling is used, which is gradually exposed to light and later, a few meshes will be opened around the groups of old beech trees, allowing the beech seedling to be installed between the spruce meshes.

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EVALUATION OF NATIVE BACTERIAL STRAINS AS PLANT GROWTH PROMOTERS FOR GREENHOUSE TOMATOES

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Abstract

Various bacterial strains have been described, over time, as plant growth promoters. The aim of this study was to evaluate the effect of some native bacterial strains applied as soil treatment on tomato seedlings, grown in greenhouse conditions, Giurgiu County. Seven bacterial strains were used in this study, three Bacillus cereus/thuringiensis strains, and one strain of each Acinetobacter guillouiae, Bacillus safensis, Paenibacillus castaneae, and P. lautus species. Certain plant growth parameters were evaluated to compare bacterial effects on the plant growth, such as leaves' chlorophyll content, plant height and stem diameter. Compared to the untreated tomato seedlings, most of the tested bacterial strains improved the plants' growth. Among all the analyzed strains, the highest chlorophyll content was registered in tomato leaves when treated with Acinetobacter guillouiae (2.07 mg/g fresh weight). The obtained results confirm the hypothesis of using selected beneficial bacteria as plant growth promoters in tomato crop. Further evaluations are needed, in order to establish the positive effects of these bacterial inoculants on tomato fruits quality and quantity.

Key words: *beneficial bacteria, native strains, plant growth promoters, seedlings, tomato.*

INTRODUCTION

Tomatoes are a widely cultivated and consumed plants across the world, with significant economic, nutritional, or cultural importance. In December 2022, the FAO reported that global tomato production in 2021, for both processing and fresh consumption, reached slightly over 189.1 million metric tons (mt). This marked a 2% increase from the 2020 production of 184.8 million mt and a 4% rise from the average production of the previous three years (2018-2020) at 182.7 million mt. Romania is one of the notable tomato producers in European Union (EU), with 9.2% of the total cultivated area (EUROSTAT, 2019). The evolution of tomato area and production cultivated in 2022, evaluated at 17170 ha and 298920 tons, was slightly decreasing compared to previous years, e.g. 2021, when surface cultivated marked 18130 ha, with a production of 500200 tons (FAO, accessed February 2024). While facing new problems in terms of agricultural technology, such as climate change, higher impact of new pests, diseases or weeds, farmers tend to use higher quantities of pesticides year by year, to respond to it. Chemical fertilizers and pesticides, while effective in boosting crop yields and controlling pests, can have detrimental effects on the environment and human health.

In the context of the increasing use of chemicals in agriculture, both worldwide and Romania, the need for new sustainable solutions has become more evident. Bacteria plant growth promoters offer a sustainable and eco-friendly alternative to synthetic chemicals. These beneficial bacteria can improve soil health and fertility (Aloo et al., 2022), enhance nutrient solubilization (Varma et al., 2020) by plants, protect crops against diseases (Aghisa et al., 2019), nitrogen fixation (N_2) (Souza et al., 2015), solubilizing potassium (Varma et al., 2019) or as phytohormones. By promoting plant growth naturally, bacteria help reduce the reliance on harmful chemicals in agriculture.

Among the most studied bacteria for their plant biostimulant proprieties, belong to genera such as genera such as *Azotobacter*, *Bacillus*, *Pseudomonas*, *Azospirillium*, and *Rhizobium* (Hurek and Reinhold Hurek, 2003; Oosten et al., 2017). Even though PGPMs (plant growth promoting microorganisms) have several advantages, their adoption in worldwide agriculture has been hindered primarily because the majority of PGPM products have low field lifetimes, mainly to their sensitive thermo-, photo- or hydro-reaction. Therefore, testing and identifying the best microorganisms as plant growth promoters, becomes an increasingly important direction in the world of research, new molecular techniques shortening the discovery process of the proprieties of these microorganisms.

MATERIALS AND METHODS

Bacterial strains

To observe growth promoting effects in tomato seedlings, 7 native bacterial strains (noted as follows, 53.3, 61.4, 59.3, 59.2S, 65.3, 50.4, LvD1) were used (Table 1).

Table 1. Native bacterial strains used in the experiment

Strain	Source isolation	Stored collection
B. cereus/thuringiensis - 53.3	Soil	RDIPP Bucharest
Paenibacillus lautus – 61.4	Soil	RDIPP Bucharest
B. safensis – 59.3	Soil	RDIPP Bucharest
Paenibacillus castanae – 59.2S	Soil	RDIPP Bucharest
B. cereus/thuringiensis – 65.3	Soil	RDIPP Bucharest
B. cereus/thuringiensis – 50.4	Soil	RDIPP Bucharest
Acinetobacter guillouaie – LvD1	Insect	RDIPP Bucharest

Bacterial strains used in the study were identified using Biolog GEN III, and some of them (50.4, 65.3) were also identified by 16S rDNA partial sequencing (Cojanu et al., 2024, Sicuia et al., 2017). Previously, the strains were growned on Luria Bertani Agar (LBA) medium (ROTH), then transferred into Erlenmeyer flask containing 50 ml of liquid Luria Bertani (LB). After inoculation the flasks were transferred to Thermoshaker, for 5 days, 120 rpm, at 30°C.

Plastic containers and substrate

Containers used in the experiment were specifically chosen (dimensions L x l x h cm, 80 x 40 x 40 cm), in order to allow the growth of 5 tomatoes seedelings per container, during the experiment observations. The soil used was universal substrate “Agro Cs”, with modified reaction, who ensures optimal aeration and accelarates root growth.

Tomato seedlings (*Lycopersicon esculentum*)

The native variety Moldoveanu F1 is known for the firm texture of fruits, resistance to diseases and for organoleptic characteristics, such as exceptional taste, vibrant red colour or sweetness.

The seedlings were obtained in controlled growth conditions (greenhouse), in special sowing pots. Randomly, for the experiment, 5 seedlings were chosen (per bacterial strain), uniform in growth, colour and with no specific disease symptoms.

In this bioassay experiment, tomato seedlings were exposed to a liquid bacteria culture to observe the effects of bacterial treatment on plant growth and development. The experiment involved three rounds of bacterial administration to the tomato seedlings.

1. ***Initial Bacterial Treatment***:

- Tomato seedlings were treated with 80 ml of liquid bacteria culture per plant. The seedlings were allowed to soak in the bacterial suspension for 2 hours to ensure thorough exposure. After the 2-hour baiting period, the seedlings were planted in soil, and the remaining liquid from the initial treatment was used to wet the seedlings equally (Figure 1a).

Figure 1. Bacterial inoculation:
a. root immersion; b. soil application

2. ***Subsequent Bacterial Treatments***:
- Following the initial bacterial administration, two more rounds of bacterial treatment were carried out. Each round involved administering 40 ml of liquid bacteria culture per plant to ensure consistent and continued exposure to the bacterial culture (Figure 1b).

Observations and Data Collection

Throughout the experiment, observations were made on the growth and development of the tomato seedlings, including leaves chlorophyll content, plant height and stem diameter.

Analysis of chlorophyll

For acquiring total chlorophyll compounds, both a and b, a systematic protocol was followed. Initially, a quantity of 1 g of tomato leaves (4 repetitions per bacterial strain) was carefully gathered and subsequently transferred to a mortar and pestle (Figure 2).

Figure 2. Tomato leaves samples for chlorophyll extraction

In conjunction, 1 g of quartz sand and 0.1 g of $CaCO_3$ were added to the mixture. The leaves were ground, and 20 ml of 100% acetone (ADRA CHIM SRL, % min 99) was poured over the resulting blend, which was then transferred to Falcon tubes.

The tubes were left undisturbed overnight at a temperature of 4°C. The following day, filtration was performed using filter paper with a weight of 389.84 g/m^2. Decimal dilutions were prepared by combining 200 microliters of the leaf mixture with 1800 microliters of acetone. Subsequently, measurements were taken using a spectrophotometer at wavelengths of 470 nm, 645 nm, and 662 nm.

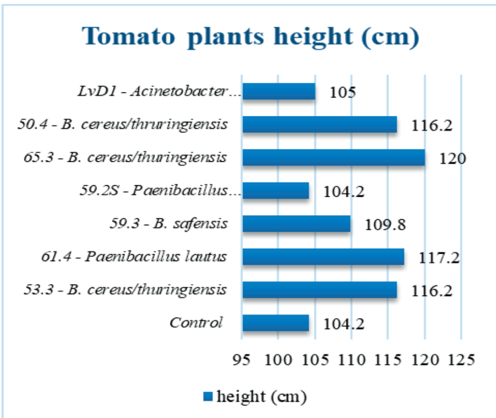
RESULTS AND DISCUSSIONS

All tested bacterial strains exhibited a positive influence on the growth of tomato plants (Figure 3). The parameters that were monitored, including plant height, stem diameter, number of leaves and chlorophyll levels, showed improvements in response to the application of the bacterial strains.

Figure 3. Tomato plants after 25 days from bacterial immersion

The **height** of the plants can serve as an indicator of their health state and overall growth. Taller tomato plants indicate vigour and a healthy growth rate. The results showed that most of the bacterial strains improved plant height, with positive values over control test (104.2 cm), from 0.2% - 104.2 cm (59.2S – *Paenibacillius castanae*) up to 15.38% - 120 cm (65.3 - *B. cereus/thuringiensis*) (Table 2).

Table. 2 Tomato seedlings height improvement with bacterial strains

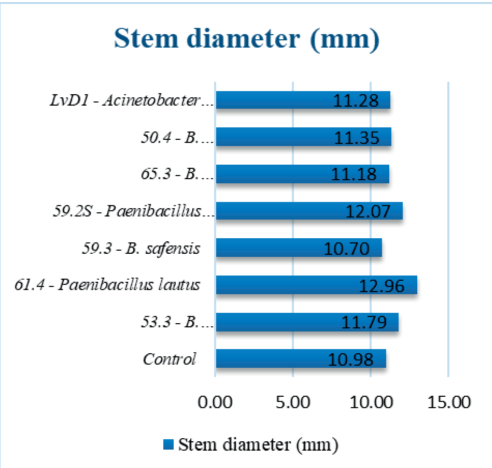


A thicker stem indicates a stronger and more robust plant. Monitoring **stem diameter**, along with other growth indicators, can help growers assess the well-being of their tomato plants and make informed decisions regarding care and maintenance. The observations were made with instrument for precise measuring (Shubler, mm), 1 cm above substrate for all plants included in test (Figure 4).

Figure 4. Stem diameter measurements

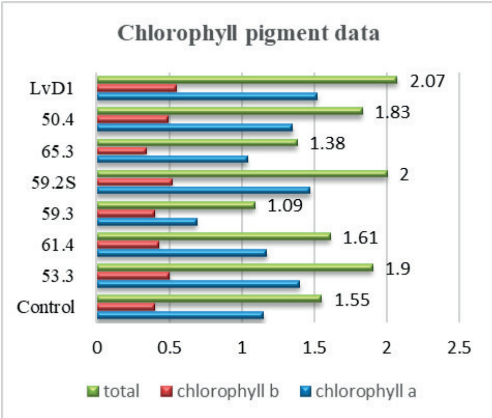
Bacterial strains used in test showed positive influence improving stem diameter values, over control (10.98 mm), from 1.8% - 11.18 mm (65.3 - *B. cereus/thuringienis*) to 18% - 12.96 mm (61.4 - *Paenibacillus lautus*) (Table 3).

Table 3. Stem diameter improvement with bacterial strains



Chlorophyll is a vital pigment found in plants that plays a vital role in photosynthesis, a or b type being the primary pigments responsible for capturing light. In tomato plants, chlorophyll and b are essential for the plant's ability to absorb and use light energy efficiently. The total amount of chlorophyll in tomato plants is an important indicator of their overall health and photosynthetic capacity. Higher levels of chlorophyll generally indicate that the plant is receiving an adequate amount of light and nutrients for optimal growth. Total chlorophyll levels are listed in Table 4.

Table 4. Total chlorophyll content analysis in tomato plants



The results suggest that different bacterial strains have varying effects on the total chlorophyll content in tomato plants. Some bacterial strains may enhance chlorophyll production, while others may have neutral impact. *Acinetobacter guillouaie* strain (LvD1), had highest total chlorophyll content compared to the control. Strains 53.3, 592S, and 50.4 also showed higher chlorophyll content than control group.

The bacterial strains involved in the test showed, for most of the followed parameters, a positive impact. Influence of plant promoting bacteria in tomato growth was analysed, research results being already reported (Adedayo et al., 2022; Gashash et al., 2022; Poria et al., 2022; Katsenios et al., 2022; Kalozoumis et al., 2021; Moustaine et al., 2017; Zaamer et al., 2016). Bacterial strains used as plant growth promoting agents improved tomato plants growth both quantitative and qualitative. The total chlorophyll content was significantly stimulated by *Acinetobacter guillouaie, Penibacillus castanae* or *B. cereus/ thruingiensis strains* comparison with other treatments and control; these results are similar to those demonstrated by Gashash et al., 2022. Comparing other test results, plant height improvement by promoting bacteria is also confirmed by other researchers (Siahaan et al., 2022).

CONCLUSIONS

Use of bacterial strains as promoting growth agents, led to improved health plant indicators, proving that the strategy of inoculation of bacteria, can represent a new tool in tomato crop technology. Better establishment of a new microorganism in the rhizosphere of a plant is directly influenced by inoculation methods. Moreover, factors like soil nutrient, pH, different soil temperatures or water should be taken into consideration, directly influencing the microorganism proprieties in plant promoting growth. Screening the influence of this factors can lead to a proper and better use of bacterial strains into an integrated management of tomato crop and more.

Further analyses are required in order to observe differences in qualitative tomato factors.

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THE EFFECT OF OPTIMIZING TECHNOLOGICAL PRACTICES ON THE INCREASE OF BIOMASS PRODUCTION AND CARBON SEQUESTRATION RATE TO *PAULOWNIA* SSP.

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Abstract

The research was carried out within an experimental batch with Paulownia elongata, established in 2019 and the fundamental purpose of the experiment was to verify the influence of the different technological links on increasing the biomass production and the carbon sequestration in young Paulownia plants. The research results demonstrated that choosing an optimum plants density of Paulownia plants at the surface unit the plants benefit from an adequate nutrition space and ensure a balanced nutrition by fertirigation during the phases considered critical for the physiological processes of vegetative growth and development, has an direct effect of stimulating the growth and development of the root system of plants and as a result the plants had a good anchoring in the soil, exploring an increasing volume of soil and realizing significantly higher biomass production and also the carbon sequestrated biomass.

Key words: *biomass production, carbon biomass, fertirigation, Paulownia ssp., plants density.*

INTRODUCTION

Originally from China, *Paulownia* ssp. has been used for more than 2500 years as an agroforestry species and in the last 50 years its cultivation has spread to all continents due to its specific characteristics related to its rapid growth rate, high ecological plasticity and its multiple uses. It was brought to Europe around the year 1800, initially as a decorative tree, after which in the last two decades it has been considered as an important commercial source, being considered as a sustainable source of biomass used for energy purposes, the production of hardwood used for the manufacture of an extremely varied range of products is of similar interest (Popescu, A. & Sabau, L., 2016; Jensen, 2016).
The name of the plant originates in Russia, in honor of the daughter of the Tsar Paul I of Russia (Woods, 2008), consort queen Anna Pavlovna, also called "Princess Tree". In ancient Japan it was a plant that was not lacking in the gardens of any family, being considered lucky, being known as the "Phoenix bird tree", due to the power of regeneration after each cut. *Paulownia*, is the solution of the future in forestry, which replaces the beautiful forests of formerly felled mercilessly due to lack, indifference or simply to enrich many. This plant dates to 1049 BC and we find it registered in Asia as a high-quality wood production for the construction of boats and houses (Kircher M., 2022). In China, about 3 million ha have been planted in the last 60 years.
Due to the abusive destruction of forests and the need for timber worldwide, Paulownia is an alternative that offers timber quality in optimum time, starting with 3-4 years for biomass (Abreu et al., 2020; Berdón et al., 2017; Woods, 2008) and 5-10 years for construction wood, which another tree species cannot offer. It absorbs very well the nitrates of the soil and the carbon dioxide in the air (Haldar & Sethi, 2021) offering freshness and tenderness through its appearance and behavior.
Due to its elasticity, it can be planted as a curtain against the wind, the blizzard on the edge of roads, airports, the protection of isolated villages from the open field (withstands over 80 km/h wind speed without breaking).

The research carried out on this "new" woody species, in the last period, had as main purpose, the creation of a clone with the best performance from the point of view of the productive potential, the parental material being quite heterogeneous and being represented only by species pure (Ols & Bontemps, 2021).
Since their initial use, the hybridization between different species has been started and the cloning with the best quantitative and qualitative performances and their official registration has been identified. As mentioned earlier, so far there are several clones available, some of which are more suitable for biomass production, while others are suitable for wood production (Fokina et al., 2020; Tyskiewicz et al., 2019).
The studies carried out so far have demonstrated the existence of a close link between the environmental conditions, the agricultural practices used and the dynamics of growth and development of different *Paulownia* species, with dry biomass production varying from the 2nd year after planting in the range of 1.5-14 t s.u./ha, the effect of agrotechnical and environmental factors also affecting the quality of wood fibers after harvesting the trees (Magar et al., 2018; Sikkema et al. 2021; Jakubowski, 2022; Muthuri et al., 2004; Gyuleva, 2008; Dubova et al., 2019).
Regarding the latter destination, even though the species has a very rapid growth, the harvesting of the stems depends on the type of wood we want to obtain, that is its size at the cubicle. From the different experiments carried out, it has been observed that the currently recommended planting scheme (4.00 m x 4.00 m), is not the one that leads to obtaining a wood of the highest quality: in fact it is known that the growth of the plant biomass is, within certain limits, directly proportional to the space in which the root system develops and the amount of solar radiation that the plant can intercept; and not only that, but also the quality of the wood would be positively influenced by the increase of these parameters (Dobrinoiu et al., 2018).
Other two factors, directly involved in obtaining superior biomass in quantitative and qualitative aspects are: optimal consumption of nutrients (macro and micro-elements) and optimal water consumption. In this context, the present research is also included, for the preparation of which a series of laboratory determinations have been made to establish the degree of influence of the planting scheme and of the fertilization scheme on the biomass production and carbon biomass sequestrated in the species *Paulownia* ssp.
The research on potential ways to reduce CO_2 emissions has demonstrated that the use of Paulownia biomass as a source of renewable energy is a viable alternative, with the constant increase in demand for wood and woody biomass already forecast in Europe until at least 2050 (Kirikkaleli et al., 2022; Hamdan & Houri, 2022; Jamil et al., 2021).

MATERIALS AND METHODS

The experience was of a bi-factorial type, placed in the field according to the method of subdivided plots; the experimental factors considered in the study being the following:
FACTOR A: planting scheme (plant density/ha), with 3 graduations:
- a_1 - Planting scheme 4.00 m x 4.00 m, resulting in a density of 625 plants/ha (Control);
- a_2 - Planting scheme 5.00 m x 5.00 m, resulting in a density of 400 plants/ha;
- a_3 - Planting scheme 6.00 m x 6.00 m, resulting in a density of 277.7 plants/ha.

FACTOR B: fertilization scheme, with 3 graduations:
- b_1 - unfertilized (Control);
- b_2 - fertilized with Polyfeed 14-14-28 + 2MgO + ME - 40 kg/ha;
- b_3 - fertilized with K-Energy Bonus (10-5-38 + 6% S + 3% Mg + B + Mn) - 40 kg/ha.

The experimental variants were placed in three repetitions, so that, following the combination of the two experimental factors, we will have an experience of the type: 3 x 3 = 9 experimental variants.
The analysis and interpretation of the experimental results was performed by the method of analysis of variance, according to the method of subdivided plots.
Each experimental variant, in order to have a constant and significant number of copies for making determinations related to physical and

mechanical properties, has the following dimensions:

a) Planting scheme 4.00 m x 4.00 m: 5 rows x 5 plants (25 plants), with an area of 400.00 m^2;

b) Planting scheme 5.00 m x 5.00 m: 5 rows x 5 plants (25 plants), with an associated area of 625.00 m^2;

c) Planting scheme 6.00 m x 6.00 m: 5 rows x 5 plants (25 plants), with an associated area of 900.00 m^2. The processing of the experimental results and their interpretation was carried out by the variant analysis method, in accordance with the experimental method of dividing the experience into subdivided plots.

Laboratory research methodology:

a) Determination of total biomass. With all the data obtained so far (air and underground biomass), the total biomass of downgraded trees will be calculated. All samples obtained from each tree have been labeled and transferred (Figure 1) to a kiln for drying.

Figure 1. Destructive sampling of Paulownia plants for biomass determination

This oven will operate at a temperature of 85°C for a period of 24 hours to dry the leaves, petioles and branches, and a temperature of 95°C for 48 hours to dry the stems and roots of each tree. After drying, they will be weighed, noting the results. To ensure that the values obtained are the final drying values, they will be placed back on the stove and left for 24 hours (Figure 2).

Figure 2. Separation of plant components and their drying for biomass determination

After 24 hours, they will be weighed again, checking whether the values will be different or not compared to those previously obtained. With these two checks, we ensure that the data obtained is real, and that the moisture losses are not lower than the initial ones because the parts are not sufficiently dry. If there were any differences between the two measurements, they would be left on the other day until the weight is constant. In this way, the dry mass of each of the components in which the trees were previously split is obtained.

b) Determination of underground biomass. To determine the root biomass, it was necessary to manually extract the root system of the plants, because the root system of the dropped trees did not have excessive dimensions.

To avoid damaging the roots of surrounding trees that have not been selected for biomass determination, a 2.00 x 2.00 m protection area around the selected tree will be provided. This square of 4.00 m^2 will be divided into 4 x 1 m^2 areas on each side to analyze the spatial distribution of fine roots around the shaft.

c) Determination of carbon biomass. Biomass carbon is obtained by multiplying total biomass of tree with default value of carbon fraction 0.47 (Eggleston et al., 2006).

RESULTS AND DISCUSSIONS

Results and discussions regarding total biomass production under experimental factors influence.

According to the determinations related to the dynamics of biomass accumulation in the different woods and organs of the *Paulownia* plants after 3 years of vegetation, a great viability is observed regarding both the biomass production achieved at the surface unit, variability that was influenced very much the density of the plants, as well as the fertilization schemes practiced in the experimental field (Tables 1-4).

Thus, the dry biomass production of Paulownia leaves related to the different experimental variants (Table 1) was between 623.93 kg/ha, the lowest biomass production obtained in the case of the control variant (a1b1), variant in which the density of plants it was 625 plants/ha, under non-fertilizing conditions and 1315.06 kg/ha. The highest biomass production was obtained when the density of the plants was 400 plants/ha, plants that were fertilized in during the vegetation period with a dose of 40 kg/ha POLYFEED 14-14-28 + 2MgO + ME (a2b2).

Table 1. Leaves and petioles carbon biomass under experimental factors influence (kg dry matter/ha)

EXPERIMENTAL VARIANT	Leaves (kg/ha)	Difference (kg/ha)	Petioles (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b1 (Control)	623.93	Control	442.93	Control	-	-
a1b2	711.68	87.75	452.78	9.85	x	x
a1b3	661.68	37.75	449.18	6.25	-	-
a2b1	1181.74	557.81	537.36	94.43	xxx	xxx
a2b2	1315.06	691.13	592.91	149.98	xxx	xxx
a2b3	1259.51	635.58	581.80	138.87	xxx	xxx
a3b1	866.21	242.28	474.53	31.60	xx	xx
a3b2	959.65	335.72	490.85	47.92	xx	xx
a3b3	939.65	315.72	484.61	41.68	xx	xx
DL5% = 53.41; DL1% = 213.57; DL0,1% = 337.68 DL5% = 7.13; DL1% = 23.51; DL0,1% = 69.33						

The biomass production of leaf petioles (Table 1) ranged from 442.93 kg/ha (a1b1) to 592.91 kg/ha (a2b2), the experimental variants in which the plant density was 400 plants/ha, in the background of 40 kg/ha fertilizer of the type POLYFEED or BONUS K-ENERGY being the most valuable from the point of view of this biometric parameter.
The branches of Paulownia plants reached a biomass production between 899.28 kg/ha and 1616.34 kg/ha, with very significant positive increases (xxx) compared to the control variant a1b1, registered in the experimental variants a2b1, a2b2, a2b3, a3b2 and a3b3, variants in which the density of the plants was 400 plants/ha and 277.7 plants/ha, respectively. The plants also benefited from the contribution of the nutrients brought after the fertilisation administration of 40 kg/ha of fertiliser (Table 2).
The biomass production of the strains, in year 3 of *Paulownia* plant vegetation, varied within quite wide limits from one experimental variant to another (Table 2), under the influence of the two experimental factors taken into consideration (planting density and fertilization scheme). Thus, under the use of a planting density of 625 plants/ha, the biomass production of the strains was between 1374.18 kg/ha and 1811.68 kg/ha. By reducing the plant density to 400 plants/ha, the production of biomass ranged from 4970.25 kg/ha to 5581.30 kg/ha while, following the use of a planting of 277.7 plants/ha, the biomass values of the strains varied between 2449.25 kg/ha and 2645.09 kg/ha. The highest values of this biometric parameter were obtained in the practice of 5.00 x 5.00 m and 6.00 x 6.00 m planting patterns. This was achieved by managing the fertilisation phase by fertilising the plants with doses of 40 kg/ha POLYFEED or BONUS K-ENERGY.
Compared to the control variant (a1b1), the experimental variants tested within the experimental field recorded production increases between 437.25 kg/ha and 4562.64 kg/ha, which statistically, were significantly positive (x) in the experimental variants a1b2 and a1b3 and very significantly positive (xxx) in the rest of the experimental variants, respectively a2b1, a2b2, a2b3, a3b1, a3b2 and a3b3. The increase of the plant nutrition space and the management of the fertilization representing the essential links in the production of super productions biomass of the stems at the surface unit.

Table 2. Branches and strains biomass production under experimental factors influence (kg dry matter/ha)

EXPERIMENTAL VARIANT	Branches (kg/ha)	Difference (kg/ha)	Strains (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b1 (Control)	899.28	Control	1374.18	Control	-	-
a1b2	1187.26	287.98	1865.43	491.25	xx	x
a1b3	1054.05	154.77	1811.68	437.50	x	x
a2b1	1258.85	359.57	4970.25	3596.07	xxx	xxx
a2b2	1616.34	717.06	5936.82	4562.64	xxx	xxx
a2b3	1585.66	686.38	5581.30	4207.12	xxx	xxx
a3b1	939.71	40.43	2449.25	1075.07	-	xxx
a3b2	1427.49	528.21	2775.65	1401.47	xxx	xxx
a3b3	1238.23	338.95	2645.09	1270.91	xxx	xxx
DL5% = 121.47; DL1% = 234.91; DL0,1% = 316.14 DL5% = 414.03; DL1% = 789.65; DL0,1% = 993.93						

Analyzing the aerial and underground biomass productions of the *Paulownia* plants, very large differences of this biometric indicator were observed (Table 3), the most valuable being the experimental variants in which the plant density was 400 plants/ha, against the background of the necessary supplementation in plant nutrients by administering 40 kg/ha POLYFEED. Thus, the *Paulownia* plants planted at a density of 625 plants/ha produced aerial biomass productions that ranged from 1965.99 kg/ha to 4217.15 kg/ha, in the experimental variants where the plant density was 400 plants/ha, the aerial biomass production ranged from 7948.20 kg/ha to 9461.13 kg/ha, the experimental variants where a planting density of 277.7 plants/ha was practiced, producing aerial biomass production between 4729.70 kg/ha and 5653.64 kg/ha.

The aerial biomass productions of the *Paulowni*a plants registered after 3 years of vegetation increases between 2010.60 kg/ha and 7495.14 kg/ha, production increases that they had, compared to the control variant (a1b1), insignificant statistical assurance (-), in the experimental variant a1b3, significantly positive (x), in the variants a1b2, a3b1 and a3b3, distinctly significant positive (xx), in the case of the a3b2 variant and very significantly positive (xxx), in the experimental variants a2b1, a2b2 and a3b2.

Following the determination of the underground biomass production it was found that as the nutrition space for the *Paulownia* plants increased the highest biomass production being obtained under the conditions of the 5.00 x 5.00 m planting scheme and the administration of 40 kg/ha POLYFEED or BONUS K-ENERGY, for these experimental variants underground biomass productions between 4681.39 kg/ha and 4970.25 kg/ha, followed by the experimental variants in which the planting scheme was practiced 6.00 x 6.00 m, these realizing underground biomass productions that varied between 2831.52 kg/ha and 3044.93 kg/ha.

Table 3. Aerial and underground biomass production under experimental factors influence (kg dry matter/ha)

EXPERIMENTAL VARIANT	Aerial biomass (kg/ha)	Difference (kg/ha)	Underground biomass (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b1 (Control)	1965.99	Control	1292.93	Control	-	-
a1b2	4217.15	2251.16	1730.43	437.50	x	x
a1b3	3976.59	2010.60	1574.18	281.25	-	-
a2b1	7948.20	5982.21	4681.39	3388.46	xxx	xxx
a2b2	9461.13	7495.14	4970.25	3677.32	xxx	xxx
a2b3	9008.27	7042.28	4803.60	3510.67	xxx	xxx
a3b1	4729.70	2763.71	2831.52	1538.59	x	xxx
a3b2	5653.64	3687.65	3044.93	1752.00	xx	xxx
a3b3	5307.58	3341.59	2945.76	1652.83	x	xxx
DL5% = 2146.72; DL1% = 3453.89; DL0,1% = 4912.44 DL5% = 356.97; DL1% = 768.91; DL0,1% = 897.63						

At the opposite pole were located the experimental variants in which the density of the plants was 625 plants/ha, the underground biomass production in their case being between 1292.93 kg/ha and 1730.43 kg/ha, well below the production level of underground biomass obtained for the other experimental variants.

Compared with the control of the experience, the rest of the experimental variants registered production increases ranging between 281.25 kg/ha and 3677.32 kg/ha, the differences regarding the underground biomass productions realized at the surface unit having a statistically insignificant positive assurance (-), in the case of the experimental variant a1b3, significantly positive (x), in the variant a1b2 and very significantly positive in the rest of the experimental variants.

The experimental factors considered in the study had a direct impact on the total biomass production realized by the *Paulownia* plants at the surface unit, productions that recorded values between 3288.92 kg/ha, the minimum value obtained in the case of the control variant (a1b1) and 1443.38 kg/ha, maximum biomass production achieved by the plants belonging to the experimental variant a2b2, variant in which the density of plants was 400 plants/ha, plants that were phased fertilized with 40 kg/ha POLYFEED (Table 4).

Taking a detailed analysis of the behavior of *Paulowni*a plants tested in different planting and fertilization schemes, we observe that, by practicing the 4.00 x 4.00 m planting scheme, biomass production ranged from 3288.92 kg/ha to 5947.58 kg/ha, between 12629.59 kg/ha and 1443.38 kg/ha, when the planting scheme was 5.00 x 5.00 m, respectively between 7561.22 kg/ha and 8698.57 kg/ha following the use of the 6.00 x 6.00 m planting scheme.

Regarding the production increases achieved by the experimental variants compared to the control of the experience, they were significantly positive (x), being between 2291.85 kg/ha and 2688.66 kg/ha, in the experimental variants a1b2 and ab3, respectively very significant positive (xxx), with production differences that varied between 4302.30 kg/ha and 11172.46 kg/ha, in the case of the other experimental variants.

Table 4. Aerial and underground biomass production under experimental factors influence

EXPERIMENTAL VARIANT	Total biomass (kg/ha)	Difference (kg/ha)	Semnificance degrees
a1b1 (Control)	3258.92	Control	-
a1b2	5947.58	2688.66	x
a1b3	5550.77	2291.85	x
a2b1	12629.59	9370.67	xxx
a2b2	14431.38	11172.46	xxx
a2b3	13811.87	10552.95	xxx
a3b1	7561.22	4302.30	xxx
a3b2	8698.57	5439.65	xxx
a3b3	8253.34	4994.42	xxx
DL5% = 2133.58; DL1% = 3349.72; DL0,1% = 4136.99			

Results and discussion regarding total carbon biomass production under experimental factors influence

The biomass of the sequestered carbon in the leaves of the *Paulownia* plants was between 293 kg/ha and 618 kg/ha, the smallest values of this parameter being recorded when using a planting density of 625 plants/ha, followed in ascending order by the variants experimental plants in which a planting density of 277.7 plants/ha was ensured, the highest amounts of organic carbon sequestered in the leaves being determined in the experimental variants where at the establishment of the plantation a plant density of 400 plants/ha was designed, with ice value they ranged from 555.41 kg/ha to 618.07 kg/ha (Table 5).

By making a difference between the experimental variants and the control variant α1b1, it is found that the accumulation of carbon in the leaves intensified with the increase of the surface of the plant foliar apparatus directly influenced by their growth force as the plants benefited from a larger space of nutrition and the optimal provision of the nutrients necessary for plant growth and development, thus eliminating the competition of plants for vegetation factors.

The differences from the experimental control were insignificant (-) in the case of experimental variant a1b3, significantly positive (x), in variant a1b2, distinctly significant positive (xx), in the variants planted according to the scheme 6.00 x 6.00 m (a3b1, a3b2, a3b3) and very significantly positive (xxx), in the experimental variants in which the planting scheme used was 5.00 x 5.00 m.

The leaf petioles sequestered an amount of organic carbon that did not show very large variations from one experimental variant, to another, the carbon biomass being between 208.17 kg/ha and 278.66 kg/ha, with differences from the control variant a1b1, which ranged from 2.94 kg/ha to 70.40 kg/ha, non-significant (-) in variants a1b2 and a1b3, significantly positive (x), in the case of experimental variants a3b1 and a3b3, distinctly significant (xx), in the variant a3b2 and very significantly positive, in the experimental variants a2b1, a2b2 and a2b3, variants in which the leaf petioles have accumulated the highest amount of carbon.

Table 5. Carbon biomass sequestreted into the leaves and petioles under experimental factors influence

EXPERIMENTAL VARIANT	Leaves (kg/ha)	Difference (kg/ha)	Petioles (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b1 (Control)	293.24	Control	208.17	Control	-	-
a1b2	334.48	41.24	212.80	4.63	x	-
a1b3	310.98	17.74	211.11	2.94	-	-
a2b1	555.41	262.17	252.55	44.38	xxx	xxx
a2b2	618.07	324.83	278.66	70.49	xxx	xxx
a2b3	591.96	298.72	273.44	65.27	xxx	xxx
a3b1	407.11	113.87	223.02	14.85	xx	x
a3b2	451.03	157.79	230.69	22.52	xx	xx
a3b3	441.63	148.39	227.76	19.59	xx	x
DL5% = 32.21; DL1% = 113.12; DL0,1% = 189.34 DL5% = 11.19; DL1% = 21.13; DL0,1% = 34.57						

Following the determination of the carbon biomass accumulated in the branches of *Paulownia* plants (Table 6), a significant increase of the values of this parameter was observed, values that varied between 422.66 kg/ha and 558.01 kg/ha, in the plants belonging to the varieties planted according to the 4.00 x 4.00 m scheme, between 441.66 kg/ha and 670.92 kg/ha, in the experimental variants in which the planting scheme was 6.00 x 6.00 m. The maximum quantities of organic carbon sequestered in the branches of the plants being determined in the case of the experimental variants in which the planting scheme used was 5.00 x 5.00 m, against the background of phase fertilization of plants with 40 kg/ha POLYFEED or BONUS K-ENERGY.

In the case of branches, compared to the control variant, the differences related to the amount of carbon sequestrated from the atmosphere varied between 19 kg/ha and 337.01 kg/ha, with statistically insignificant positive assurance (-), in variants a1b3 and a3b1, distinctly significant positive (xx), in the case of variant a3b1 and very significantly positive (xxx), in the rest of the experimental variants.

Table 6. Carbon biomass sequestreted into the branches and strains under experimental factors influence

EXPERIMENTAL VARIANT	Branches (kg/ha)	Difference (kg/ha)	Strains (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b1 (Control)	422.66	Control	645.86	Control	-	-
a1b2	558.01	135.,35	876.75	230.89	xx	-
a1b3	495.40	72.74	851.48	205.73	-	-
a2b1	591.65	168.99	2336.01	1690.15	xxx	xxx
a2b2	759.67	337.01	2790.30	2144.44	xxx	xxx
a2b3	745.26	322.60	2623.21	1977.35	xxx	xxx
a3b1	441.66	19.00	1151.14	505.28	-	xx
a3b2	670.92	248.26	1304.55	658.69	xxx	xxx
a3b3	581.96	159.30	1243.19	597.33	xxx	xxx
DL5% = 116.68; DL1% = 122.13; DL0,1% = 151.13 DL5% = 312.17; DL1% = 443.88; DL0,1% = 562.16						

Analyzing the results regarding the sequestrated carbon biomass in the constituent organs of the *Paulownia* plants, it was demonstrated from the research that the highest amount of organic carbon was retained in the plant strains (Table 6), the carbon biomass in this case showing a strong variability between the 9 experimental variants taken in study. Thus, at the plant density of 625 plants/ha, the biomass of sequestered carbon ranged from 645.86 kg/ha to 876.75 kg/ha, by reducing the plant density to 400 plants/ha, the carbon biomass varied between 2336.01 kg/ha and 2790.30 kg/ha while at a density of 277.7 kg/ha the carbon biomass was between 1151.14 kg/ha and 1304.55 kg/ha.

The differences registered in this parameter, compared to the control of the experience (a1b1), were insignificant (-), in the experimental variants a1b2 and a1b3, distinctly significant positive (xx), the variant a3b1 and very significantly positive, in the rest of the experimental variants analyzed.

The total aerial biomass of the sequestered carbon recorded values between 924.01 kg/ha and 4446.73 kg/ha, the plants tested under the conditions ensuring a planting density of 400 plants/ha and fertilized at doses of 40 kg/ha fertilized totally soluble and fully assailable to plants by fixing the highest amounts of organic carbon (Table 7). Differences from the control variant ranged from 944.98 kg/ha to 3522.72 kg/ha, with statistically insignificant positive assurance (-) for experimental variants a1b2 and a1b3, distinctly positive (xx), for variants a3b1 and a3b3 and very significant positive (xxx), in experimental variants a2b1, a2b2, a2b3 and a3b2.

Table 7. Total aerial and underground carbon biomass sequestreted under experimental factors influence

EXPERIMENTAL VARIANT	Aerial biomass (kg/ha)	Difference (kg/ha)	Underground biomass (kg/ha)	Difference (kg/ha)	Semnificance degrees	Semnificance degrees
a1b2	1982.06	1058.05	813.30	205.63	-	x
a1b3	1868.99	944.98	739.86	132.19	-	-
a2b1	3735.65	2811.64	2200.25	1592.58	xxx	xxx
a2b2	4446.73	3522.72	2336.01	1728.34	xxx	xxx
a2b3	4233.88	3309.87	2257.69	1650.02	xxx	xxx
a3b1	2222.95	1298.94	1330.81	723.14	xx	xx
a3b2	2657.21	1733.20	1431.11	823.44	xxx	xx
a3b3	2494.56	1570.55	1384.50	776.83	xx	xx
DL5% = 1136.82; DL1% = 1245.91; DL0,1% = 1617.83; DL5% = 186.23; DL1% = 477.71; DL0,1% = 1184.95						

The carbon biomass resulting from the analysis of the root system of plants was between the limits of 607.67 kg/ha and 813.30 kg/ha, by practicing the planting scheme of 4.00 x 4.00 m, between the limits of 1330.81 kg/ha and 1431.11 kg/ha, when using the planting scheme of 6.00 x 6.00 m, the most valuable being the plants harvested from the experimental variants in which the planting scheme was 5.00 x 5.00 m, these having a well-developed root system, with great capacity to explore a volume high soil and thus fixing a large amount of organic carbon (Table 7).

Compared with the control variant (a1b1) in which the carbon biomass was 607.67 kg/ha, the rest of the experimental variants recorded differences from insignificantly positive (-), to variant a1b3, significantly positive (x), to a1b2, distinctly significant positive (xx), in the experimental variants a3b1, a3b2 and a3b3, up to very significant positive (xxx) in the experimental variants a2b1, a2b2 and a2b3.

Table 8. Total carbon biomass sequestrated under experimental factors influence

EXPERIMENTAL VARIANT	Total biomass (kg/ha)	Difference (kg/ha)	Semnificance degrees
a1b1 (Control)	1531.69	Control	-
a1b2	2795.36	1263.67	x
a1b3	2608.86	1077.17	-
a2b1	5935.90	4404.21	xxx
a2b2	6782.74	5251.05	xxx
a2b3	6491.57	4959.88	xxx
a3b1	3553.77	2022.08	x
a3b2	4088.32	2556.63	xxx
a3b3	3879.06	2347.37	xx
DL5% = 1163.18; DL1% = 2331.12; DL0,1% = 2471.91			

Compared to the control variant (a1b1), in the other 8 experimental variants (Table 8), there were registered increases in the total biomass of carbon sequestered by the plants, increases that had a statistically insignificant positive assurance (-), in variant a1b3, significantly positive (x), in experimental variants a1b2 and a3b1, distinctly significant positive (xx), in the case of variant a3b3 and very significantly positive (xxx), in experimental variants a2b1, a2b2, a2b3 and a3b2, variants in which the *Paulownia* plants were characterized by increased capacity for assimilation and fixation of organic carbon in the atmosphere.

CONCLUSIONS

Overall, it is observed that the biomass production of the leaves increased directly in proportion to the increase of the nutrition space related to the plants, but also with the dose of fertilizer administered to them during the vegetation period.

Thus, the biomass production of the petioles showed increases compared to the control of the experience with the same statistical assurance as with the biomass of the leaves, there being a direct correlation between the two components of the leaf, respectively between the tongue and their petiole.

The biomass production of the branches increased directly in proportion to increasing the distance between plants and the dose of fertilizer administered to the plants by fertilization during their vegetation period, the highest values of this biometric parameter being obtained by practicing the two 5.00 x 5.00 m, respectively 6.00 x 6.00 m planting schemes.

The biomass of the stems was directly influenced by the density of the plants at the surface unit and by the administration of the phase fertilization of the *Paulownia* plants, at the planting density of 400 plants/ha, respectively 277.7 plants/ha, the production increases exceeding the control production with over 1075.07 kg/ha.

By ensuring a planting density of 400 kg/ha and ensuring a balanced nutrition with macro and micro-elements essential for the growth and harmonious development of *Paulowni*a plants, superior aerial biomass production was achieved, with very significant production increases of between 5982.21 kg/ha and 7495.14 kg/ha.

The administration of POLYFEED type fertilizers at a dose of 40 kg/ha and ensuring optimum planting density significantly influenced the total biomass production of *Paulownia* plants, benefiting from both a balanced intake of nutrients and a proper nutrition station, factors that stimulated the physiological processes of plant growth and development in good conditions, which led to the recording of significantly higher total biomass production increases, while also increasing the value of biomass as a raw material in the wood industry.

Carbon biomass sequestered in the leaves of 3-year-old *Paulownia* plants, recorded the lowest value in terms of planting density of 625 plants/ha, in conditions of non-fertilization of plants, biomass that increased with the reduction of plant density to surface unit and with additional administration of nutrients by fertilizing plants with fertilizer doses of 40 kg/ha.

The highest amount of sequestered carbon in the *Paulownia* plant branches (759.67 kg/ha) was determined under the conditions of using an optimum density of 400 plants/ha and administering during the vegetation period of plants 40 kg/ha POLYFEED.

Carbon biomass sequestered in the stems of *Paulownia* plants, after 3 periods of their vegetation, reached maximum values when at planting a density of 400 plants/ha was provided, against the background of supplementing the necessary nutrients by phasing the plants with 40 kg/ha POLYFEED.

Of the planting schemes, respectively the fertilization schemes, tested in the experience with *Paulownia*, the most effective in terms of the ability of plants to sequester organic carbon from the atmosphere and to achieve higher values of carbon biomass has proved to be the planting scheme that it provided a density of 400 plants/ha, against the background of fertilizing plants with doses of 40 kg/ha POLYFEED, respectively 40 kg/ha BONUS K-ENERGY.

Under the conditions of planting density of 277.7 plants/ha, the total carbon biomass increased significantly, compared with the values obtained in the variants where the plant density was maximum (625 plants/ha) whereas, compared with the experimental variants where the number of plants per unit area was 400 plants/ha, there was a decrease in the ability of plants to assimilate organic carbon from the atmosphere, which gives us the possibility that the greatest impact on the dynamics of carbon sequestration in particular.

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RESEARCH ON THE EVOLUTION OF THE PHYSICAL AND MECHANICAL PARAMETERS OF *PAULOWNIA* FIBER UNDER DIFFERENT PLANTING AND FERTILIZATION CONDITIONS

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Abstract

Two factors are directly involved in obtaining superior wood in quantitative and qualitative aspects, respectively an optimal consumption of nutrients (macro and micro-elements) and the optimal plants density at the surface unit. In this context, the present research have been made in order to establish the influence degree of the planting and the fertilization scheme on the physical and mechanical properties that restore the value from the point of view of wood quality to Pauownia ssp. Experimental results showed that best of all the fibers harvested from the experimental variants where the planting schemes of 5x5 m or 6x6 m were used and were administered 40 kg/ha Polyfeed 14-14-28+2MgO+ME by fertirigation during the vegetation period of the plants, the balanced ratio between the nutrients giving to the plants increased resistance to the mechanical factors action.

Key words*: Paulownia ssp., plants density, fertirigation, fiber's quality, physical parameters, mechanical parameters.*

INTRODUCTION

The *Paulownia* tree originates in Asian countries (China, Vietnam), and is still widely cultivated in East Asia. The *Paulownia* genus, included in the Paulowniaceae family by the latest genetic research, includes several species, between 6 and 12 depending on the accepted taxonomic classification, the best known species being *Paulownia tomentosa*, *P. elongata* and *P. fortunei* (Jakubowski, 2022). The tree was brought to Europe at the beginning of the 19th century, imported by the Dutch East India Company, the name "paulownia" or "princess tree" being given in honor of Queen Anna Pavlovna of the Netherlands, daughter of Tsar Paul I of Russia. In the last decades, Paulownia was introduced in more than 40 countries, from various continents: North America, Australia, South America, etc.

Although initially *Paulownia* was cultivated in Europe as an ornamental tree, today it is used for many other purposes, as a result of the many interesting characteristics of this plant and the advantages of the culture (Jiménez & Rodríguez, 2003). The irrational deforestation at the planetary level in the last decades has serious consequences regarding the increase in carbon emissions and visible climate changes (Hamdan et Houri, 2022). Many countries, including the EU, have launched several initiatives to reduce carbon emissions, in which forests play an essential role, being large CO_2 sinks (Haldar and Shethi, 2021). Due to the rapid growth, *Paulownia* tree culture can be a very efficient solution for reforestation projects, including the very high rate of carbon absorption, due to the well-developed leaf system (40 tons of CO_2/ha/year) (Jensen, 2016).

On the other hand, thanks to the very well developed root system, the culture of these trees can protect the land against soil erosion and the stabilization of landslides, even contributing to improving the quality and fertility of the soil, thanks to the ability to fix nitrogen (Gyuleva, 2008).

Due to the rapid growth, the *Paulownia* culture is suitable for the production of biomass (Jose et Bardhan, 2012), used not only as a source of energy (biofuels), but also as a use of wood, the growth of the biomass of a *Paulownia* plantation being higher by about 30% compared to poplar and willow, e.g. *Paulownia* wood is much lighter compared to other

species, having an average density of 260 kg/m^3 (Barbu et al., 2022).
Paulownia is the solution of the future in forestry, which replaces the beautiful forests of formerly felled mercilessly due to lack, indifference or simply to enrich many (House et al., 2002). This plant dates back to 1049 BC and we find it registered in Asia as a high quality wood production for the construction of boats and houses. In China, about 3 million ha have been planted in the last 60 years. Due to the abusive destruction of forests and the need for timber worldwide *Paulownia* is an alternative that offers timber quality in optimum time, starting with 3-4 years for biomass and 5-10 years for construction wood, which another tree species cannot offer (Dobrinoiu et al., 2018; Haldar and Sethi, 2021). It absorbs very well the nitrates of the soil and the carbon dioxide in the air, offering freshness and tenderness through its appearance and behavior. Due to its elasticity it can be planted as a curtain against the wind, the blizzard on the edge of roads, airports, the protection of isolated villages from the open field (withstands over 80 km/h wind speed without breaking) (Kaymakci et al., 2013).
The wood has a great capacity for modeling and handling, being able to be used in the furniture industry (as solid wood or veneer) or in the manufacture of musical instruments, these being known by instrument manufacturers in Asia as having special acoustic properties. The wood can be successfully used in the timber industry, being resistant to rotting and termite attack, due to the fact that it is rich in tannins. The low weight of *Paulownia* wood makes it very suitable for the manufacture of light wooden structures, for example for aircraft structures or for other parts where weight is essential (Kaymakci et al., 2013). The research carried out on this "new" woody species, in the last period of time, had as main purpose, the creation of a clone with the best performance from the point of view of the productive potential, the parental material being quite heterogeneous and being represented only by species pure (Fokina et al., 2020).
So far there are several clones available, some of which are more suitable for biomass production, while others are suitable for wood production (Dubova et al., 2019).
Regarding the latter destination, despite the fact that the species has a very rapid growth, the harvesting of the stems depends on the type of wood we want to obtain, that is its size at the cubicle (Koman et al., 2017).
The physical-mechanical properties of the wood of the same species can vary by up to 30% and these variations can be considered frequent (Jakubowski, 2022), for these it is recommended to study the properties of a wood for each area of origin, cutting age determining its quality.

MATERIALS AND METHODS

The experiement was carried out in a 5-year-old *Paulownia* plantation in the specific soil-climatique condition of Tulcea county and the fundamental purpose of the experiment was to verify the influence of the different technological links (planting density and fertilization scheme) on increasing the technological characteristics of the wood obtained at harvest.
The objectives of the research were:
- determination of the physical properties of the fibers belonging to *Paulownia* plants aged 5 years;
- determination of the mechanical properties of the fibers belonging to *Paulownia* plants aged 5 years.

The exeperiment been placed in the field in a bifactorial experience type, in subdivided plots with three repetitions, the experimental factors tested in the study being the following:
FACTOR A: plants density at the unit surface, with the follow graduations:
- a1 - equidistant planting at 4.00 m x 4 m, with a density of 625 plants per hectare (Control);
- a2 - equidistant planting at 5.00 m x 5.00 m, with a density of 400 plants per hectare;
- a3 - equidistant planting at 6.00 m x 6.00 m, with a density of 277.7 plants per hectare.

FACTOR B: scheme of fertilizing, with the follow graduations:
- b1 - unfertirigated (Control);
- b2 - fertirigated with 40 kg/ha Polyfeed 14-14-28 + 2MgO + ME;
- b3 - fertirigated with 40 kg/ha Bonus K-Energy (10-5-38 + 6% S + 3% Mg + B + Mn).

The analysis and interpretation of the experimental results was performed by the method of analysis of variance, according to the method of subdivided plots.

Table 1. The dimensions of the samples according to the norm

Species properties			Height (mm)	Amplitude (mm)	Length (mm)
Moisture content by drying		UNE EN 13183-1:03			
Moisture content by resistance hygrometer		UNE EN 13183-2:03			
Specific weight		UNE 56 531:77	20	20	25 ±
Hygroscopicity		UNE 56 532:77	20	20	40
Linear and volumetric contraction		UNE 56 533:77	20	20	40
Determination of hardness		UNE 56 534:77	20	20	40
Determination of axial compression		UNE 56 535:77	20	20	60
Resistance to dynamic bending		UNE 56 536:77	20	20	300
Resistance to static bending		UNE 56 537:77	20	20	300
Tensile strength perpendicular to fiber	radial	UNE 56 538:78	20	20	70
	tangential	UNE 56 538:78	20	20	70
Splitting resistance		UNE 56 539:78	20	20	70
Resistance to perpendicular compression on fibers		UNE 56 542:88	50	50	150

In order that the values obtained in the characterization are comparable with the results of other tests, the methodology used was indicated in the norm tested (Table 1).
The determinations regarding the physical and mechanical properties of the fibers were made with small samples, without defects that harm the values of a certain property of the wood.
In order to determine the size of the specimens, the criterion of the Spanish norm described in the UNE norms was followed, which uses specimens with the section dimensions of 20 x 20 mm^2 for most physical and mechanical tests, except for the resistance to the cutting force and compression perpendicular to the fiber, for which the norms of the UNE establish samples with 50 x 50 mm^2 section.
The samples were collected from the experimental group, from *Paulownia* plants aged 5 years, using destructive plant samples, separately for each experimental variant.
In order to obtain the specimens, with the dimensions specified in the regulations in force, the stems were cut in the laboratory workshop, according to the specific method for determining each physical and mechanical parameter followed.
The specimens needed to test the physical-mechanical properties of *Paulownia* fibers were obtained from the 3-year-old plant stems, from a unit of 60 cm in length, from which the specimens were subsequently made, by sectioning to the dimensions provided in the regulations according to Figure 1.
The pieces contain marrow and young wood were eliminated being cut in half, resulting in slabs with a section of 2 x 2 cm^2 and the axis parallel to the axis of the trunk. From these chips were obtained the test pieces used to test the physical and mechanical parameters of the fiber.
Also, specimens with a section of 5 x 5 cm^2 and 15 cm in length were obtained on which tests were performed on fiber resistance to perpendicular compression and parallel cutting (splitting).
The physical tests were performed on 20 x 20 x 40 mm samples, in which the weights and linear dimensions (radial, tangential and longitudinal) were measured, under different humidity conditions. From the obtained values, the physical and mechanical properties were calculated, according to the specific standard of each determined parameter.

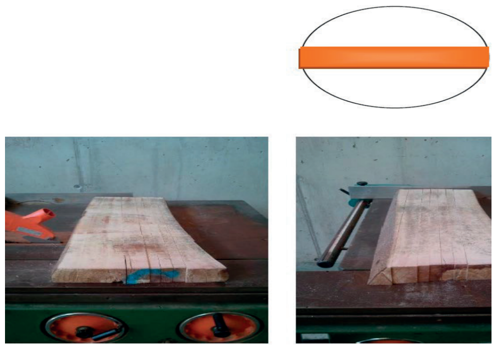

Figure 1. Preparation of the specimens for determination of physical-mechanical parameters

Determination of moisture content of fiber by drying: UNE EN 13183-1: 03. The protocol of work: Weigh the specimen, then dry it at a temperature of 103 ± 2ºC until the difference between two successive weighings at intervals of 2 hours is less than 0.1%. Re-sample the sample (Figure 2).

Equipment needed to determination: SCALTEC Digital Scale 2200 X 0.01gr (CET.LAB.003.01); 72 l SELECTA THEROVEN 2000W cooker (CET.LAB.005.01); 300 mm crystal dryer (CET.LAB.006.01)

Figure 2. Determining of specific humidity and weight

Determination of specific weight: UNE 56 531: 77. The protocol of work: Weigh the specimen, measure its size and then calculate it to obtain the specific weight in g/cm^3. Repeat the procedure in anhydrous state. Equipment needed to determine: SCALTEC Digital Scale 2200 X 0.01gr (CET.LAB.003.01); 72 l SELECTA THEROVEN 2000W cooker (CET.LAB.005.01); 300 mm crystal dryer (CET.LAB.006.01)

Determination of fiber hygroscopicity: UNE 56 532: 77. The protocol of work: It is calculated from the values of the coefficient of volumetric contraction and the specific weight of the sample.

Determination of linear and volumetric fiber contraction: UNE 56 533: 77 20. The protocol of work: Fully insert the specimen into the water, at room temperature, until it exceeds the saturation humidity, after which the dimensions of the specimen are measured and its volume is calculated. Leave the specimen at room temperature until it reaches the hygroscopic equilibrium after which again the dimensions of the specimen are measured and its volume is calculated. Dry the specimen at a temperature of 103 ± 2ºC until the difference in weight between two successive weighing at 2-hour intervals is less than 0.1% (Figure 2). Measure the sample size in anhydrous state and calculate its volume. Equipment needed to determine: SCALTEC Digital Scale 2200 X 0.01gr (CET.LAB.003.01); Digital jack (S225) (CET.LAB.004.01); 72 l SELECTA THEROVEN 2000W cooker (CET.LAB.005.01); 300 mm crystal dryer (CET.LAB.006.01)

Determination of fiber hardness: UNE 56 534: 77.

Figure 3. Determining the physical hardness of the fiber

The protocol of work Place the specimen in the weight machine with the radial part up, then operate the load until it reaches 100 kg per cm width, in the case of very soft timber until it reaches 50 kg and multiply by 2, the deformation obtained (Figure 3). The Hardness is calculated and the Hardness Rate is obtained. Equipment needed to determine: Weight machine (CET. LAB.010.01).

Determination of fiber resistance to linear compression: UNE 56 535: 77. The protocol of work: Place the lumber specimen in the weight machine, with the longitudinal direction of the fibers perpendicular to the plates of the weight press. Apply the weight at a uniform rate from 200 to 300 kg/cm^2 per minute, until breaking (Figure 4).

Figure 4. Determining of fiber resistance to linear compression

The resistance to axial compression is calculated. Equipment needed to determine: Weight machine (CET. LAB.010.01).

Determination of fiber resistance to dynamic bending: UNE 56 536: 77. The protocol of work: Place the specimen in the weight machine, at a distance of 24 cm between the supports, with the radial side up.

Figure 5. Determining of fiber resistance to bending

The hammer of the machine falls from a height of 1000 mm ± 1 mm (Figure 5). The dynamic bending resistance is then calculated. Equipment needed to determine: Weight machine (CET. LAB.010.01).

Determination of tensile strength perpendicular to fiber: UNE 56 538: 78. The protocol of work: Fix the specimen between the traction machine guides and then apply traction weight, until it breaks (Figure 6). Thereafter, the tensile strength perpendicular to the fiber and the adhesion rate are calculated. Equipment needed to determine: Weight machine (CET. LAB.010.01).

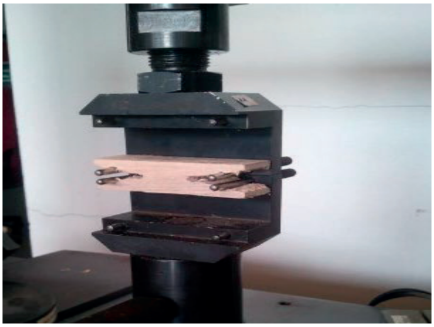

Figure 6. Determining of the tensile strength perpendicular to the fiber

Determination of fiber resistance to splitting: UNE 56 539: 78. How to work: The specimen is fastened between the trusses (jaws) of the traction machine and then the traction weight is applied until breaking. Split resistance and breaking rate are calculated. Equipment needed to determine: Weight machine (CET. LAB.010.01).

Determination of resistance to perpendicular compression of fibers: UNE 56 542: 88. How to work: Place the specimen in the weight machine. The tensile weight is applied until it is broken and the resistance to perpendicular compression of the fiber is calculated. Equipment needed to determine: Weight machine (CET. LAB.010.01).

RESULTS AND DISCUSSIONS

Results and discussion regarding physical characteristics of *Paulownia*'s fibers

Analyzing the quality of *Paulownia* fibers, in terms of their density at 12% humidity (Table 2), due to the influence of the two experimental factors studied (planting density and fertilization scheme), it is observed that the values of this physical parameter of quality registered values between 209 kg/m^3, minimum value obtained in the case of experimental variant a2b2, variant in which the planting scheme was 5x5 m, the plants benefiting during the vegetation period by the contribution of 40 kg/ha POLYFEED 14- 14-28+2MgO+ME and 257 kg/m^3, maximum value recorded for the experimental variant in which the planting scheme of 4x4 m was practiced, under non-fertilization conditions (a1b1-Control).

By increasing the nutrient space of the plants, supported by the optimal supply with their nutrients, an inversely proportional increase of the fiber density is observed. Thus, if we make a comparison between the experimental variant taken as a control (a1b1) and the rest of the experimental variants, there are recorded differences that varied between -6 kg/m^3 and -48 kg/m^3, with statistically insignificant assurance (-), to the a3b1 variant, significantly negative (o), at a2b1 and very significantly negative (ooo), at the rest of the experimental variants.

The same situation is found after determining the anhydrous and saturated density of the analyzed *Paulownia* fibers. Thus, the values of anhydrous fiber density ranged from 187 kg/m^3 (a2b2) to 224 kg/m^3 (a1b1-Control), the values of this physical indicator increasing directly in proportion to the decrease of the plant nutrition space and decreasing inversely with the increase the distance between plants and the administration of fertilizers.

Table 2. Fiber density, as a result of the combined action of experimental factors

Experimental Variants	Density at 12% (kg/m^3)	Difference (kg/m^3)	Anhydrous density (kg/m^3)	Difference (kg/m^3)	Saturated density (kg/m^3)	Difference (kg/m^3)	Semnification		
a1b1 (Control)	257	Control	224	Control	445	Control	-	-	-
a1b2	217	-40	198	-26	413	-32	ooo	ooo	ooo
a1b3	229	-28	209	-15	428	-17	ooo	oo	o
a2b1	245	-12	221	-3	416	-29	o	-	oo
a2b2	209	-48	187	-37	385	-60	ooo	ooo	ooo
a2b3	227	-30	192	-32	403	-42	ooo	ooo	ooo
a3b1	251	-6	219	-10	438	-7	-	-	-
a3b2	213	-44	197	-27	407	-38	ooo	ooo	ooo
a3b3	222	-35	212	-12	426	-19	ooo	o	o
DL5% =9.3; DL1% = 13.6; DL0.1% = 21.4 DL5% =11.2; DL1% = 18.7; DL0.1% = 25.6 DL5% =15.1; DL1% = 24.3; DL0.1% = 31.3									

The difference from the control variant was between -3 kg/m^3 (a2b1) and - 37 kg/m^3 (a2b2), with statistically insignificant assurance (-) in the case of experimental variants (a2b1 and a3b1), significantly negative (o), in the variant a3b3, distinctly significant negative to the experimental variant a1b3 and very significantly negative (ooo), to the rest of the experimental variants tested within the experiment. The saturated fiber density had values between 385 kg/m^3, the minimum value obtained under the conditions of the 5 x 5 m planting scheme, against the background of fertilization with Polyfeed 14-14-28+2MgO+ME, at a dose of 40 kg/ha and 445 kg/m^3 (a2b2), maximum recorded in the case of the control variant (a1b1), with the differences recorded being between -7 kg/m^3 and -60 kg/m^3, having insignificant statistical assurance (-), in the variant a3b1, significantly negative (o), at a1b3 and a3b3, distinctly significant negative (oo), at a2b1 and very significantly negative (ooo), for the rest of the experimental variants analyzed.

Table 3. The hygroscopicity of the fibers, as a result of the combined action of the experimental factors

Experimental Variants	Hygroscopicity (kg/m^3)	Difference (kg/m^3)	Semnification
a1b1 (Control)	1.29	Control	-
a1b2	1.37	0.08	xx
a1b3	1.54	0.25	xxx
a2b1	1.25	-0.04	o
a2b2	1.07	-0.22	ooo
a2b3	1.32	0.03	x
a3b1	1.31	0.02	-
a3b2	1.13	-0.16	ooo
a3b3	1.42	0.13	xxx
DL5% =0.03; DL1% = 0.05; DL0.1% = 0.11			

From the point of view of fiber hygroscopicity (Table 3), there was a great variability of the values of this physical parameter between the 9 experimental variants taken in the study, values that varied from 1.07 kg/m^3, in the case of the experimental variant in which planting was done according to the planting scheme 5x5 m, the plants benefiting from phase fertilization with Polyfeed 14-14-28+2MgO+ME, at a dose of 40 kg/ha (a2b2) and 1.54 kg/m^3, maximum value of to this indicator obtained by the mature variant where the planting scheme of 4 x 4 m was practiced, under conditions of non-fertilization of the plants (a1b1). Overall, it was observed that the hygroscopicity of *Paulownia* fibers decreases inversely with the increase of the plant nutrition space, with differences from the control variant, statistically insured, very significant negative (ooo) in the variants a2b2 and a3b2, significantly negative (o), at a2b1, insignificant (-), at variant a3b2, significantly positive (x), for variant a2b3, distinctly significant positive (xx), at experimental variant a1b2 and very significantly positive (xxx) at variants a1b3 and a3b3.

The same variability between the experimental variants was also recorded after determining the degree of contraction of the fibers (Table 4). Thus, the volumetric contraction of the fibers was between 7.7% and 9.2%, by practicing the planting scheme of 4 x 4 m, 6.7% and 7.9% in the case of the 5 x 5 m planting scheme, respectively between 7.1% and 8.8% by using the 6 x 6 m planting scheme, the smallest values of this physical indicator being recorded under conditions of non-fertilization of the plants, regardless of the planting density.

Table 4. Fiber contraction as a result of the combined action of experimental factors

Experimental Variants	Volumetric contraction (%)	Difference (%)	Linear contraction (%)	Difference (%)	Semnification	
a1b1 (Control)	7.7	Control	4.2	Control	-	-
a1b2	8.6	0.9	4.7	0.5	xxx	xxx
a1b3	9.2	1.5	5.1	0.9	xxx	xxx
a2b1	6.7	-1.0	3.8	-0.4	ooo	ooo
a2b2	7.1	-0.6	4.1	-0.1	ooo	ooo
a2b3	7.9	0.2	4.3	0.1	ooo	xxx
a3b1	7.1	-0.6	4.1	-0.1	ooo	ooo
a3b2	8.2	0.5	4.5	0.3	xxx	xxx
a3b3	8.8	1.1	4.8	0.6	xxx	xxx
DL5% =0.01; DL1% = 0.03; DL0.1% = 0.05 DL5% =0.02; DL1% = 0.04; DL0.1% = 0.06						

Analyzing *Paulownia* fibers in terms of resistance to linear contraction, we obtained values of this physical parameter of fiber quality ranging from 4.2% to 5.1% by using the 4x4 m planting scheme, between 3.8% and 4.3%, when the planting was done at a distance between 5x5 m plants and between 4.1% and 4.8% after practicing the 6 x 6 m planting scheme, with differences compared to the control variant that had statistical assurance from very significant negative (ooo), in the case of experimental variants a2b1, a2b2, a3b1, to very significant positive (xxx), to the rest of the experimental variants tested in the experiment. The values of the fiber hardness indices (Table 5) showed a very high variability between the experimental variants taken into study, both under the influence of planting density and under the aspect of plant supply in nutrients, with indices between 0.02 and 0.15, the lowest hardness level being recorded after planting at a distance of 5 x 5 m, under conditions of non-fertilization (a2b1).

Compared with the control variant (a1b1), the other experimental variants recorded very significant negative differences (ooo), in the experimental variants a2b1, a2b2, a2b3, a3b1, significantly negative (o), in the a3b2 variant, significantly positive (x), at variant a1b2, distinctly significant positive (xx), in the case of variant a3b3 and very significantly positive (xxx), in the experimental variant a1b3. The radial hardness at the dimension of the *Paulownia* fibers tested had values between 2.09 and 2.16, under the conditions of the 4 x 4 m, 1.79 and 1.95 planting scheme, following the 5 x 5 m planting scheme, respectively between 1.96 and 2.12 by planting at a distance of 6 x 6 m, with differences from the control which had very negative statistical assurance, in the case of experimental variants a2b1, a2b2, a2b3 and a3b1, insignificant (-), for experimental variants a1b2 and a3b2, significantly positive (x), for variant a3b3 and distinctly positive (xx), in the experimental variant a1b3.

Table 5. The radial hardness of the fibers, as a result of the combined action of the experimental factors

Experimental Variants	Radial hardness (index)				Semnification	
	N	Difference	Share	Difference		
a1b1 (Control)	0.09	Control	2.09	Control	-	-
a1b2	0.12	0.03	2.11	0.02	x	-
a1b3	0.15	0.06	2.16	0.07	xxx	xx
a2b1	0.02	-0.07	1.79	-0.3	ooo	ooo
a2b2	0.04	-0.05	1.83	-0.26	ooo	ooo
a2b3	0.07	-0.02	1.95	-0.14	ooo	ooo
a3b1	0.05	-0.04	1.96	-0.13	ooo	ooo
a3b2	0.10	0.01	2.08	-0.01	o	-
a3b3	0.12	0.03	2.12	0.03	xx	x
DL5% =0.01; DL1% = 0.03; DL0.1% = 0.05 DL5% =0.03; DL1% = 0.06; DL0.1% = 0.09						

Results and discussion regarding the mechanical characteristics of *Paulownia*'s fibers.

The two experimental factors studied (planting density and fertilization scheme) significantly influenced the mechanical characteristics of the *Paulownia* fibers subjected to the analyzes, with a high variability of them (Table 6). Thus, following the determination of the axial compression of the fibers belonging to the strains harvested from the 9 experimental variants taken in the study, it was found that at the humidity of 12% of the samples values were recorded that varied between 193 kg/cm^2, minimum value recorded in the case of the strains from the experimental variant a2b2 (5x5 m, fertilized with 40 kg/ha Polyfeed 14-14-28+2MgO+ME) and 223 kg/cm^2 in the case of the control variant (4x4 m, unfertilized), with differences from the control which they had very significant negative insurance (ooo), under the conditions of the 5 x 5 m planting scheme, regardless of the fertilizer administered.

This, it justifies us to affirm that, from the point of view of this parameter, the nutritional status of the *Paulownia* plants had a direct impact on the values recorded by this parameter of fiber quality. In the experimental variant a3b2, the differences from the control variant (a1b1) were significantly negative (o) whereas, the rest of the experimental variants have insignificant statistical assurance (-) compared to the control variant.

The influence of the fertilization scheme on the degree of axial compression of the fibers was even more evident when this mechanical parameter was determined at the level of the static quota of the fibers, the values obtained following the determinations varying within quite wide limits, respectively from 7.8 kg/cm^2, under the conditions of administration of 40 kg/ha Polyfeed 14-14-28 + 2MgO + ME, against the background of the planting scheme 5x5m (a2b2), up to 8.8 kg/cm^2, under the conditions of the control variant in which it was planted 4 x 4 m planting scheme, under conditions of non-fertilization of plants (a1b1). Compared with the control variant (a1b1), the rest of the experimental variants recorded very significant negative differences (ooo), except for the experimental variants in which the *Paulownia* plants were planted at a distance of 4 x 4 m, variants where the differences were insignificant (-), respectively significantly negative (o).

Table 6. Axial fiber compression as a result of the combined action of experimental factors

Experimental Variants	Axial compresion (kg/cm^2)		Difference (kg/cm^2)		Semnification	
	Value at 12%	Static share	Value at 12%	Static share		
a1b1 (Control)	223	8.8	Control	Control	-	-
a1b2	216	8.4	-7	-0.4	-	o
a1b3	219	8.7	-4	-0.1	-	-
a2b1	211	8.1	-12	-0.7	ooo	ooo
a2b2	193	7.8	-30	-1.0	ooo	ooo
a2b3	203	7.9	-20	-0.9	ooo	ooo
a3b1	221	8.3	-2	-0.5	-	ooo
a3b2	214	7.9	-9	-0.9	o	ooo
a3b3	217	8.1	-6	-0.7	-	ooo
DL5% = 7.39; DL1% = 12.64; DL0.1% = 16.82 DL5% =0.21; DL1% = 0.46; DL0.1% = 0.64						

The results of the determinations regarding the behavior of *Paulownia* fibers at dynamic and static bending are centralized in Table 7.

The resistance of Paulownia fibers to mechanical bending recorded values between 0.09 kp/cm^2 and 0.20 kp/cm^2, under planting conditions at a distance of 4 x 4 m, between 0.06 kp/cm^2 and 0.11 kp/cm^2, in following the planting at 5 x 5 m and between 0.08 kp/cm^2 and 0.19 kp/cm^2 by practicing the 6 x 6 m planting scheme, best behaving at the action of the mechanical bending forces the specimens collected from the experimental variants where the distance between plants were 5 and 6 m respectively and the plants benefited during the vegetation period from a balanced supply with nutrients (a2b2, a2b3 and a3b2), nutrients that significantly increased the elasticity of the fibers.

Table 7. The bending resistance of the fibers, as a result of the combined action of the experimental factors

Experimental Variants	Dinamic bending (kp/cm^2)	Difference (kp/cm^2)	Static bending at 12% (kp/cm^2)	Difference (kp/cm^2)	Semnification	
a1b1 (Control)	0.20	Control	371	Control	-	-
a1b2	0.09	-0.11	352	-19	ooo	ooo
a1b3	0.18	-0.02	365	-6	o	o
a2b1	0.11	-0.09	348	-23	ooo	ooo
a2b2	0.06	-0.14	337	-34	ooo	ooo
a2b3	0.09	-0.11	345	-26	ooo	ooo
a3b1	0.19	-0.01	369	-2	o	-
a3b2	0.08	-0.12	348	-23	ooo	ooo
a3b3	0.14	-0.06	362	-9	o	oo
DL5% =0.01; DL1% = 0.04; DL0.1% = 0.07 DL5% =4.39; DL1% = 7.64; DL0.1% = 8.89						

The differences, compared to the variant taken as an experimental control (a1b1) had a statistically significant negative assurance (o), in the case of the experimental variants a1b3, a3b1 and a3b3, differences that became very significantly negative (ooo) in the case of the samples from the experimental variants. which plant density was 400 plants/ha and 40 kg/ha were administered Polyfeed 14-14-28+2MgO+ME, respectively Bonus K-Energy (10-5-38+6%S+3%Mg+B+Mn), the fibers showing in these situations increased elasticity.

Regarding the resistance of the fibers to the static bending, at their 12% humidity, the recorded values ranged from 337 kp/cm^2 to 371 kp/cm^2, with differences compared to the very negative control variant (ooo) in the experimental variants a1b2, a2b1, a2b2, a2b3 and a3b3, distinctly significant negative (oo), a3b3, significant negative (o), a1b3 and insignificant (-), in the experimental variant a3b1.

Table 8. The tensile strength of the fibers, as a result of the combined action of the experimental factors

Experimental Variants	Fiber traction at 12%				Semnification	
	Tangential (kp/cm^2)	Difference (kp/cm^2)	Radial (kp/cm^2)	Difference (kp/cm^2)		
a1b1 (Control)	10.91	Control	11.23	Control	-	-
a1b2	10.81	-0.10	11.17	-0.06	ooo	ooo
a1b3	10.83	-0.08	11.19	-0.04	oo	oo
a2b1	10.82	-0.09	11.18	-0.05	ooo	oo
a2b2	10.67	-0.24	11.07	-0.16	ooo	ooo
a2b3	10.71	-0.20	11.11	-0.12	ooo	ooo
a3b1	10.94	0.03	11.20	-0.03	xx	o
a3b2	10.86	-0.05	11.15	-0.08	oo	ooo
a3b3	10.88	-0.03	11.19	-0.04	oo	oo
DL5% =0.02; DL1% = 0.03; DL0.1% = 0.09 DL5% =0.02; DL1% = 0.04; DL0.1% = 0.06						

From a statistical point of view, compared to the control variant (a1b1), there were very significant negative differences (ooo) in the experimental variants a1b2, a2b1, a2b2 and a2b3, distinctly significant negative (oo), in the variants a1b3, a3b2, and a3b3, distinctly significant positive (xx), in the experimental variant a3b1, variant in which fibers with lower tensile strength were obtained, compared to the rest of the experimental variants.

The same situation was founded when was analyzed the results of the determinations related to the fiber resistance to radial traction, the values of this parameter being between 11.07 kp/cm^2 and 11.23 kp/cm^2 (Table 8), the fibers of the highest quality, in terms of their resistance to mechanical actions, being obtained under the conditions of fertilization of plants with a dose of Polyfeed 14-14-28+2MgO+ME, regardless of the density of the plants, the differences from the control of the experience (a1b1) having a very negative statistical assurance (ooo).

CONCLUSIONS

In terms of fiber density, the lightest wood, with high quality fibers, was obtained under the conditions of planting schemes 5x5m and 6x6 m, against the background of 40 kg/ha Polyfeed 14-14-28+2MgO+ME and BONUS K-ENERGY (10-5-38+6%S+3%Mg+B+Mn).

The hygroscopicity of *Paulownia* fibers increased directly in proportion to the reduction of the nutrition space of the plants, regardless of the type of fertilizer administered to the plants during the vegetation period, fibers of the highest quality being obtained on the basis of the administration of 40 kg/ha Polyfeed 14-14-28+2MgO+ME under the conditions of the 5x5 m planting scheme.

The volumetric and linear contraction of the fibers increased directly in proportion to the increase of the plant nutrition space and the quantity of nutrients available to the plants in order to stimulate the physiological processes of growths and their development.

The radial hardness of the fibers increased directly in proportion to the dose of fertilizer administered during the vegetation period of the *Paulownia* plants and conversely proportional to the increase in the available nutrition space to the plants.

By achieving a planting density of 400 plants/ha (5x5 m) and ensuring a balanced nutrition with nutrients of *Paulownia* plants, the mechanical properties of the fibers, in particular the elasticity and the resistance of the fibers to dynamic and static bending, are significantly improved.

The tensile strength of the fibers was influenced to a lesser extent by the planting scheme used in the experimental field, having a direct impact on the fiber quality from the point of view of this indicator having the optimum supply of plants throughout the vegetation period with the indispensable nutrients their growth and development, elements that are easily accessible to plants, easily assimilated and in a balanced ratio.

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COMPARISON OF THE MICROBIAL COMMUNITY OF ORCHARD SOILS IN THE NORTH-EAST PART OF ROMANIA

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Abstract

Orchard crop species can influence the soil microbial population by releasing root exudates, which are compounds released by plant roots into the soil. Some crop species exude compounds that are more beneficial for certain groups of microbes, while others exude compounds that inhibit the growth of certain groups. Additionally, different crop species have different root systems, which can affect the physical structure of the soil and the availability of water and nutrients, which in turn can influence the soil microbial population. This study aimed to determine the soil microbial diversity of some orchards during different seasons to advance knowledge about the role of microbes in the balance of the orchard ecosystem. The research was carried out in a mixed orchard in Iasi county. Effects of season (i.e. autumn, spring and summer) and land use (apple orchard, plum orchard and cherry orchard) on soil microbial populations and microbial community structures were determined using the Petri dish culture method on different culture media.

Key words: *soil microbiome, soil microbial community, orchard soil.*

INTRODUCTION

Fruits are among the most attractive and delicious horticultural species, and together with vegetables, they represent important components for a healthy and balanced diet (Bvenura & Sivakumar, 2017; Mandal et al., 2021). The fruit tree species, such as apple, plum, or cherry trees, can exert various influences on the soil in which they are cultivated and on the physical, chemical, and biological properties of the soil (Montanaro et al., 2017). Among the physical properties of the soil, those strongly influenced by fruit trees are represented by soil structure, soil temperature, soil aeration, as well as soil erosion (Letey, 1985; Van Quang et al., 2012). The roots of fruit trees directly influence the soil structure, thereby affecting soil porosity and facilitating the circulation of air and water around the roots. Additionally, the well-developed root system of fruit trees reduces the risk of soil erosion. Fruit trees also influence soil temperature due to the shading provided by their canopy, which helps maintain soil temperature at a lower value during hot periods, thus avoiding soil overheating in the summer (Pregitzer et al., 2000). Besides the physical and chemical properties, soil undergoes a series of biological changes when fruit tree species are cultivated. Fruit trees consume significant amounts of essential nutrients from the soil (such as nitrogen, phosphorus, potassium), leading to changes in nutrient concentrations in the soil and potentially influencing their availability to soil microorganisms (Qian et al., 2014). Another important aspect is the chemicals secreted by the roots of fruit trees into the soil, leading to changes in the soil environment near the roots through modifications in pH, the presence of acids, or other substances that can influence soil microorganisms (Si et al., 2018). Additionally, fruit trees also influence the biological properties of the soil through interactions established between soil microorganisms and the roots of fruit trees or as a result of the indirect influence exerted by the roots of trees or the modification of the physical and chemical properties of the soil.

The substances secreted by plant roots into the soil can serve as food for various groups of soil microorganisms, stimulating the growth and activity of soil microbiota (Hayat et al., 2010). Certain species of fruit trees are capable of forming symbiotic relationships with nitrogen-fixing bacteria (e.g. actinorhizal plants with *Frankia* spp.). Fruit trees can contribute to increasing the biodiversity of soil microorganisms through various ecological interactions established between fruit trees and soil microbiota (Schwintzer, 2012; Van Nguyen & Pawlowski, 2017).

Understanding the soil microbiota of fruit trees is important due to the multiple roles microorganisms play, such as decomposing organic matter, protecting plants against diseases and pests, and enhancing soil fertility through nutrient cycling (Mercado-Blanco et al., 2018; Castellano-Hinojosa & Strauss, 2020).

Given the aforementioned factors, soil samples were collected from a mixed orchard of apple, plum, and cherry trees between April and September 2023 to assess the influence these three fruit tree species have on the soil microflora.

MATERIALS AND METHODS

The research sites were established in an orchard (apple, plum and cherry) at S.C. Agralmixt S.A. farm (Figure 1), located in the North-Eastern Romania, Iasi county, Andrieseni village ($47^0$34'9" N, $27^0$20'38" E, 60 m above sea level).

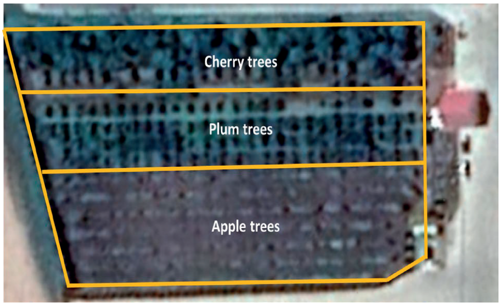


Figure 1. Orchard within the farm S.C. Agralmixt S.A.

The climate of this region is a temperate continental with an average annual air temperature and precipitation of 9.5°C and 520 mm, respectively (Gafencu et al., 2023; Nazare et al., 2023).

The average air temperature and precipitation during the sampling months were 8.7°C and 99.8 mm in April, 20.6°C and 36.4 mm in June, and 19.5°C and 23.2 mm in September (Figure 2).

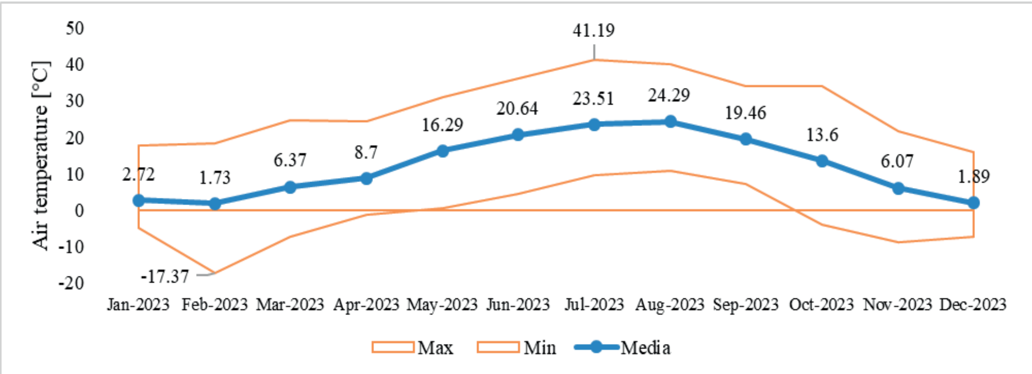


Figure 2. The air temperature (average, minimum and maximum monthly) recorded in the year 2023 within the farm S.C. Agralmixt S.A. at weather station 00001CED

The average monthly air temperature, in the studied area, did not register negative values during the analyzed period, but during the winter months the daily air temperature drops below the 0°C threshold. The lowest value of the air temperature was -17.37°C, recorded on February 10, 2023. In the summer months, the average monthly air temperature exceeds 20°C, in the July-August period there were days when air temperature values over 40°C were recorded, for example July 2 and 4, 2023 (Figure 3).

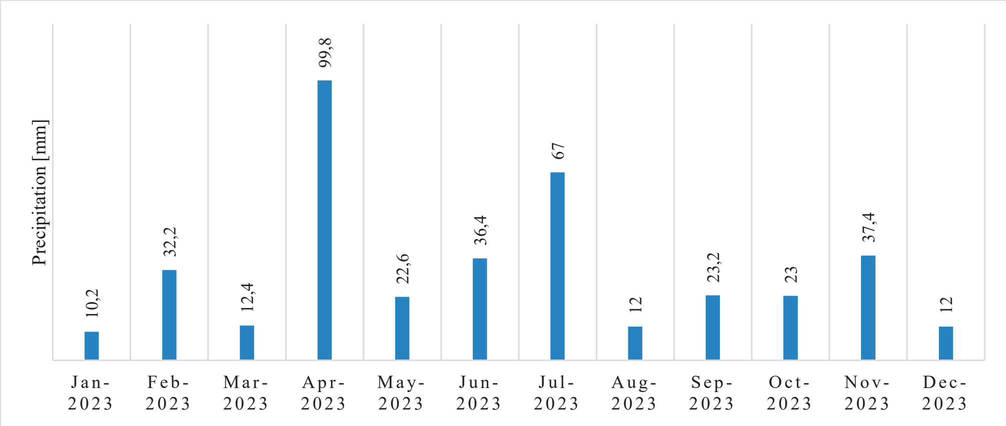


Figure 3. The monthly amount of atmospheric precipitation recorded in 2023 within the S.C. Agralmixt S.A. farm at weather station 00001CED

At the Agralmixt farm, in the orchard, within the framework of applied technology, large amounts of chemical fertilizers and pesticides are not used. Treatments are carried out only in case of strong attacks by pathogens or pests.

Soil samples were collected separately for each species (apple, plum, and cherry), representing the analyzed plots (Figure 3).

The soil samples were collected at three different times (April, June, and September 2023). Within each plot, samples were taken from 10 randomly selected points. The soil was collected from near the roots of the fruit trees, at a depth of about 10 cm.

The soil samples were transported to the Microbiology Laboratory of the Iasi University of Life Sciences for preparation, where they were stored overnight at 4°C, dried at room temperature, and sieved before undergoing microbiological analysis.

To determine the total number of bacteria in the soil, expressed as CFUs (colony-forming units) per gram of dry soil, the culture method in Petri plates was employed, involving a series of serial dilutions prior to plating (Gafencu & Ulea, 2023). To determine the total number of bacteria in the soil, the Potato Dextrose Agar (PDA) culture medium (Scharlau, Spain, 39 g l^{-1}) was used (Ulea et al., 2017). Streptomycin (35 mg l^{-1}) was incorporated into the media to inhibit the growth of Gram-negative (G^-) bacteria, thereby enabling the enumeration of Gram-positive bacteria (G^+). Streptomycin was thoroughly mixed into the PDA media post-autoclaving (15 minutes at 121°C), at a temperature of approximately 48°C. For the assessment of filamentous fungi, the PDA medium was also employed, with the addition of Bengal Rose at a concentration of 35 mg l^{-1}. The Rose Bengal was used to limit the size of faster-growing molds (Smith & Dawson, 1944).

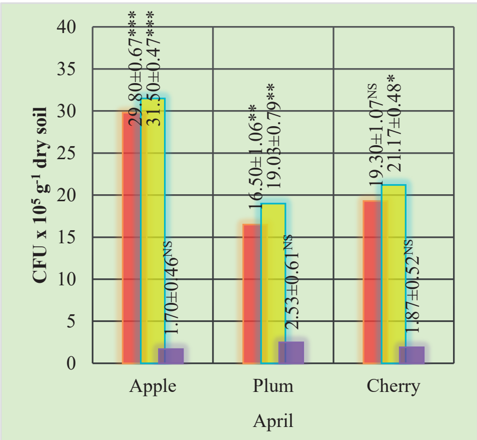
For each sample, the method of successive dilutions was employed. To determine bacteria and fungi in the soil, one ml of the suspension from the 10^{-3} and 10^{-4} dilutions, respectively, was transferred to a Petri dish. Subsequently, 17 ml of media were added at a temperature of 48°C and homogenized by orbital movements. After solidification of the culture media, the Petri dishes were incubated at a temperature of 28°C. After 24 hours, the number of bacterial colonies that developed was determined for both the PDA media and the PDA media containing Streptomycin. The Scan® 1200 automatic colony counter (Interscience, France) was used to determine the number of colonies that developed.

To determine the total number of bacterial colonies per gram of soil, the values obtained from each analyzed Petri dish were multiplied by the inverse value of the dilution factor. The results were expressed in colony forming units per gram of dry soil (CFU x 10^5 g^{-1} dry soil). Filamentous fungi were assessed after 5 days, and identification was based on morphological characteristics (Gilman, 1957; Barnett, 1960; Ellis & Ellis, 1985; Seifert & Gams, 2011; Guarro et al., 2012). Fungi that did not form spores during the 5-day period and could not be identified were grouped together (Other species). Statistical analysis of the data obtained in the experiments was performed using the SPSS program (IBM SPSS Statistics 26). For the statistical analysis of the data, a benchmark represented by the average value was utilized for comparison with the obtained values. Additionally, mean values were determined for each sampling moment and for each parameter, including the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria.

RESULTS

Some recent studies show that fruit species such as apple, plum or cherry can influence the soil microbiota (Franke-Whittle et al., 2015; Si et al., 2018).

The data obtained from the conducted research indicate that in April, the total number of bacteria in soil sampled from the apple orchard was significantly higher compared to soil sampled from plum and cherry orchards (Figure 4).



Total number of bacteria – Yellow bars
Gram positive bacteria (G^+) – Purple bars
Gram negative bacteria (G^-) – Red bars

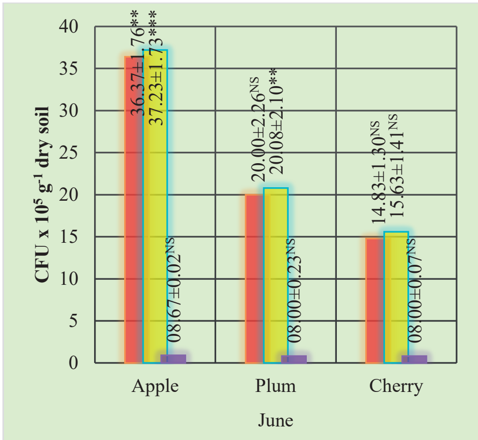
NS – $P > 0.05$
* – $P \leq 0.05$
** – $P \leq 0.01$
*** – $P \leq 0.001$

Figure 4. Soil bacterial communities under the influence of tree species - in April 2023

According to the results, in the apple orchard, the total number of bacteria was 31.50±0.47 CFUx10^5g^{-1}dry soil, whereas in soil sampled from the plum and cherry orchards, the total number of bacteria was 19.03±0.79 CFUx10^5 g^{-1} dry soil and 21.17±0.48 CFUx10^5g^{-1} dry soil, respectively.

In the case of Gram-negative bacteria, the same trend was observed regarding the number of bacteria in the soil, with significant differences noted between the values obtained from soil sampled from the apple orchard compared to soil sampled from the cherry and plum orchards. However, concerning Gram-positive bacteria, the data obtained indicate that in April, the number of Gram-positive bacteria in soil sampled from the apple orchard was lower compared to the number of Gram-positive bacteria identified in soil sampled from the cherry and plum orchards.

Following the second sampling in June, it was observed that the number of bacteria in the soil increased in the apple and plum orchards, while in the cherry orchard, the number of bacteria decreased (Figure 5).



Total number of bacteria – Yellow bars
Gram positive bacteria (G^+) – Purple bars
Gram negative bacteria (G^-) – Red bars

NS – $P > 0.05$
* – $P \leq 0.05$
** – $P \leq 0.01$
*** – $P \leq 0.001$

Figure 5. Soil bacterial communities under the influence of tree species - in June 2023

Following this sampling, it was observed that the apple orchard recorded the highest values of the total number of bacteria in the soil, specifically measuring 37.23±1.73 CFUx10^5g^{-1} dry soil. In the case of the cherry orchard, a total bacterial count of 15.63±1.41 CFUx10^5g^{-1} dry soil was determined.

Towards the end of the vegetative period of the fruit tree species, the number of soil bacteria slightly decreased in all three orchards. At this sampling moment, the highest bacterial count was still observed in the soil sampled from the apple orchard, with a count of 31.07±6.50

CFUx10^{5}g^{-1} dry soil (Figure 6). At every sampling moment and across all three species, it was observed that the majority of soil bacteria are Gram-negative, constituting approximately 95% of the total bacterial population in the soil.

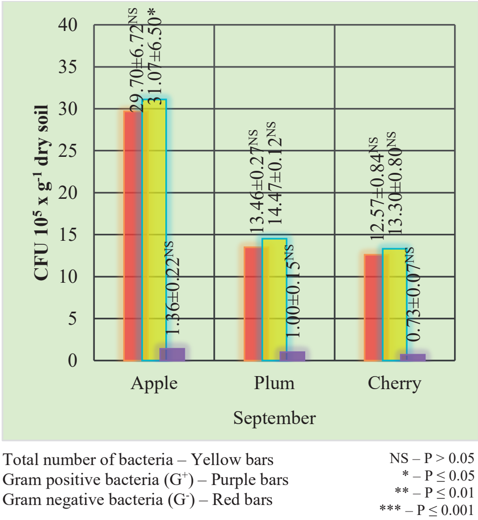


Figure 6. Soil bacterial communities under the influence of tree species - in September 2023

The ratio between Gram-positive and Gram-negative bacteria in soil is an important aspect of microbial diversity in soil. Generally, the majority of bacteria in soil are Gram-negative, with their proportion often exceeding 90% of the total bacterial population.

However, Gram-positive bacteria also play a crucial role in the soil ecosystem, contributing to nutrient cycles and organic matter decomposition processes. The balance and diversity between these two types of bacteria are crucial for soil health and its proper functioning. The findings concerning filamentous fungi capable of cultivation on synthetic culture media reveal that morphological assessments identified a total of 10 genera of micromycetes in the soil samples. However, certain fungi remained unidentified due to their lack of spore production during the 5-day observation period, thus being classified under the group labelled as "Other species" (Figure 7).

Predominantly, the most abundant colonies of fungi developed on the PDA with Rose Bengal culture media belonged to the *Penicillium* genus. Over half of the fungi colonies that developed on the culture media belonged to this genus. This trend was consistent across all three species and in all three sampling moments, with one exception noted in the plum orchard, where *Penicillium* represented 35.90% of the total identified micromycete genera.

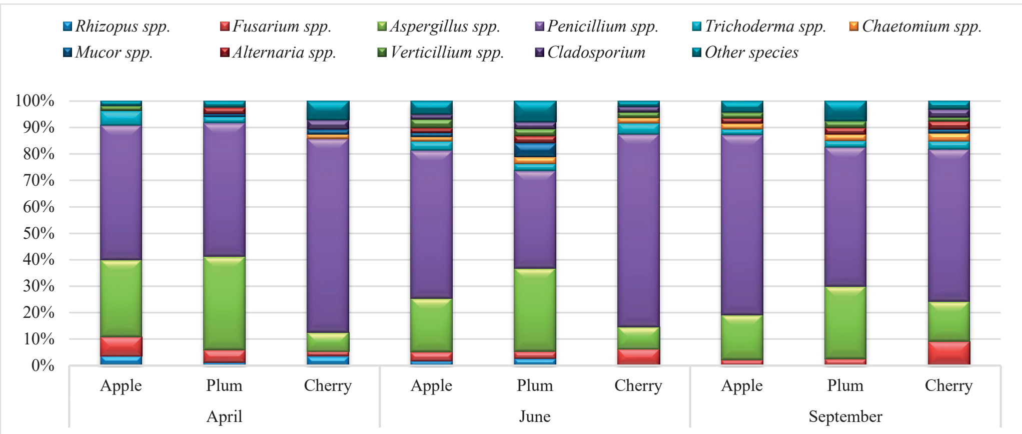


Figure 7. Changes of soil fungi structure during the vegetation period of fruit trees

The second most prevalent genus of micromycetes was *Aspergillus*. This genus represented between 7% and 35% of the fungal colonies that developed on the culture media. Analyzing the number of colonies developed in Petri dishes revealed that approximately 75-80% of the developed colonies belonged to these two genera. In addition to *Penicillium* and *Aspergillus*, other fungi belonging to the genera *Rhizopus*, *Fusarium*, *Trichoderma*, *Chaetomium*, *Mucor*, *Alternaria*, *Verticillium*, and *Cladosporium* were identified on the culture media. Additionally, there were fungi that could not be identified based on morphological characteristics (either due to sterile mycelium or lack of spore formation within the 5-day observation period). All other identified genera represented between 1% and 9% of the total micromycetes identified.

CONCLUSIONS

The results obtained from the research conducted throughout the year 2023 on the soil microbiota of the three pomological species (apple, plum, and cherry) indicate a higher number of bacteria in soil sampled from the apple orchard compared to the other two species. This suggests a significant difference in soil microbiota depending on the fruit tree species. It is possible that this observation is linked to specific soil conditions and requires further investigation to understand the underlying reasons for this discrepancy.

Regarding the micromycetes in the soil, it was observed that the majority of micromycetes belong to the genera *Penicillium* and *Aspergillus*. However, alongside these, other genera of fungi with high importance for the biological processes in soil are also present.

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VALUABLE COMPOUNDS DERIVED FROM CUCURBITACEAE SUBPRODUCTS AND POTENTIAL WAYS OF ITS VALORIZATION

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Abstract

The paper shows a short documentary study regarding the main valuable compounds that are present in the sub-products resulting in the fruits from the Cucurbitaceae family, with a main accent on Cucurbita sp. type pumpkins. The compound types (polyphenolic compounds, triterpenoids, carotenoidic compounds), extraction methods and the properties of bioproducts obtained in this way (antimicrobial, antitumor properties, sun protection factor) are presented. The most suitable valorization methods for waste biomass generated by Cucurbitaceae fruits are the following: 1) recovery of the carotenoids and polyphenolics compound from these, with potential applications in obtaining different dermato-cosmetical formulations, and 2) obtaining of nanomaterials with silver and gold, mediated by the phyto-compounds recovered by extraction from Cucurbitaceae peels, with potential applications in medicine.

Key words*: antioxidant activity, antimicrobial effect, Cucurbita sp., peel, pumpkin, waste.*

INTRODUCTION

The family Cucurbitaceae belongs to the order Cucurbitales and contains 98 genera and about 975 species of edible and ornamental plants.
This family of plants includes cucumbers, gourds, melons, and squashes. Members can be annual or perennial, and they can grow in temperate or tropical climates on Earth (Encycolpedia Britannica, 2024).
Cucurbitaceae of interest for the food industry and not only, are *Citrullus* sp. (watermelon, citron), *Cucumis* sp. (cantaloupe), *Cucumber* sp. (cucumbers), *Cucurbita* spp. (squash, pumpkins), *Trichosanthes cucumerina*, *Sechium edule*, *Benincasa hispida*, *Coccinia grandis*, *Momordica charantia* (Botany Illustrated, 2006; Vieira et al., 2019, Perez Gutierrez, 2016). Due to their widespread cultivation and usage in Romania, the by-products of pumpkins (*Cucurbita* sp.) are the subject of this study.

CHEMICAL COMPOSITION OF PUMPKIN

The species belonging to this family are rich in carotenoids, terpenoids, saponins, alkaloids, anthocyanins, tannins, and polyphenolic compounds (Rolnik et al., 2020; Harith et al., 2018).
Pumpkins are rich in vitamins, α, β, γ-carotene, lutein, violaxanthin, and neoxanthin, as well as amino acids and proteins that have antifungal activities (Mukherjee et al., 2022; Zaharie et al., 2022).
The fruits peel of *Cucurbita sp.* contains macroelements (Figure 1), microelements (Figure 2) and nutrients such as proteins, carbohydrates, and lipids (Figures 3-4).

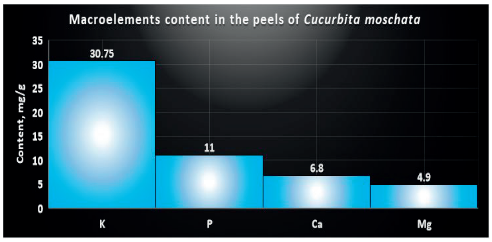


Figure 1. Macronutrient content in *C. moschata* peels (adapted after Salehi et al., 2019)

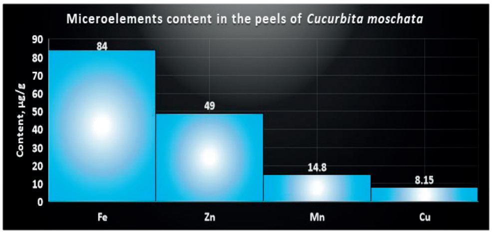


Figure 2. Micronutrient content in *C. moschata* peels (adapted after Salehi et al., 2019)

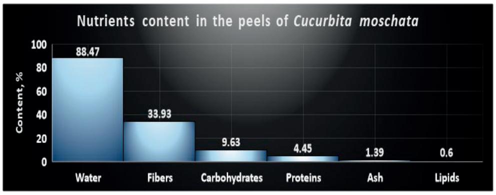


Figure 3. Nutrients content in *C. moschata* peels (adapted after Salehi et al., 2019)

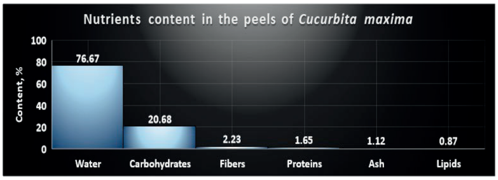


Figure 4. Nutrients content in *C. maxima* peels (adapted after Salehi et al., 2019)

Cucurbita maxima (pumpkin) is an important source of vitamin A (Ragasa and Lim, 2005; Bawara et al., 2010; Abou-Zaid et al., 2001); B vitamins (thiamine, riboflavin, niacin, folic acid), C, D, F, E vitamins are also found in the fruits of other species of Cucurbitaceae, such as *Lagenaria siceraria*, *Trichosanthes cucumerina* (snake squash) (Shah and Seth, 2010; Adebooye, 2008), *Coccinia indica* (ivy gourd) (Sachin and Trisa, 2018). Other plants belonging to the Cucurbitaceae family, such as *Cucurbita maxima* (pumpkin), *Luffa acutangula* (angled loofah, Chinese Okra) contain small amounts of vitamin K and E (Avinash and Rai, 2017). Chemical substances possessing insecticidal and antibacterial properties are prevalent in these species (Rajasree et al., 2016; Yadav et al., 2010; Jamuna et al., 2015). Studies have demonstrated that the whole extract functions better than the individual compounds, while the exact mechanisms of action remain unclear (Salehi et al., 2019). In a study by Cheong et al. (1997) it was demonstrated that the 8 kDa molecular mass protein derived from *Cucurbita moschata* has antifungal properties *in vivo*. Experiments conducted *in vitro* using a protein extracted from this species, known as the PR-5 protein, demonstrated that it strongly suppresses the growth of *Candida albicans* and *Fusarium oxysporum* (Ramalhete et al., 2011). The methanolic extract of *Citrullus lanatus* peels (Harith et al., 2018) inhibited the development of some microorganisms such as *S. epidermidis* and *T. mentagrophytes*, at concentrations of 20 mg extract/mL. The extracts derived from multiple varieties of pumpkins demonstrated antimicrobial activity against the intestinal flagellate parasite *Giardia lamblia* and microorganisms of the types of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella spp.* or *Klebsiella spp.*, and *Vibrio cholerae* (Table 1). According to research of Salehi and colab. (Salehi et al., 2019), the pumpkin peel extracts show antifungal action against the microorganisms such us: *Fusarium* sp., *Trichoderm* sp., *Aspergillus* sp., *Verticillium* sp., *Phytophora* sp., *Botrytis* sp., *Candida* sp., and *Saccharomyces*.

Table 1. Biological effects of bioproducts obtained from pumpkin fruits and/or peels

Cucurbita spp. Plant part	Solvent	Biological effect	Microorganism/cell line	References
Cucurbita moschata Duchesne, crude proteins obtained from peels	acetone	antimicrobial	*Aspergillus fumigatus* *Aspergillus parasiticus* *Aspergillus niger; Staphylococcus aureus* *Bacillus subtilis; Klebsiella pneumoniae* *Pseudomonas aeruginosa; E. coli*	Elhadi et al., 2013
Cucurbita maxima Duchesne, pulp	petroleum ether and methanol	vermifuge	*Giardia lamblia*	Muruganantham et al., 2016
Cucurbita maxima Duchesne, peels	aqueous	antimicrobial	*E.coli;Pseudomonas sp.;Vibrio cholerae*	Kabbashi et al., 2014
Cucurbita moschata		antitumoral	Standardized human tumor cell lines: Hela, HCT-8, HepG-2	Feng et al., 2019
		antitumoral	Standardized type tumor cell lines: K562, B16, A549	Hou et al., 2008
		antidiabetic	Asian patients with diabetes mellitus	Salehi et al., 2019
		antifungal antiproliferative, antiulcer	*Fusarium oxysporum; Candida albicans*	Cheong et al., 1997 Abdel-Rahman, 2006; Gill et al., 2011
Cucurbita ficifolia (pulp)		antidiabetic and antioxidant	Male rats	Xia și Wang, 2007
		treatment for type 2 diabetes	Hyperglycemic rabbits	Roman-Ramos et al., 1992; Acosta-Patino et al., 2001
Cucurbita andreana		antitumoral, anti-inflammatory		Jayaprakasam et al., 2003

EXTRACTION METHODS OF BIOACTIVE COMPOUNDS FROM *CUCURBITA* SP. PEELS

According to Kulczyński et al. (2020), ideal extraction techniques typically involve a mass ratio of dry plant to solvent of 1:10, a maximum temperature of 70°C, and an extraction duration of two hours.

Pumpkin peel extracts that may be utilized straight into cosmetic formulations can be produced by using an ecological extraction process and "green solvents" aqueous solutions (Figure 5), aqueous solution of propylene glycol 20% (Figure 6), or aqueous solution of ethanol 70% (Figure 7).

Investigations performed *in vitro* on normal standardized human cell lines (keratinocytes), have demonstrated that extracts of this kind do not exhibit cytotoxicity at concentrations below 1000 µg/mL.

Given their high flavonoid content, notable antioxidant activity, and promising sun protection factor, the aqueous extracts were the most promising (Gaweł-Bęben et al., 2022; Indrianingsih et al., 2019).

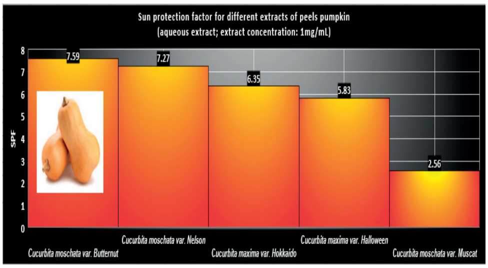


Figure 5. The sun protection factor for aqueous extracts obtained from peels of different pumpkin varieties (crude extract concentration = 1 mg/mL) (adapted from Gaweł-Bęben et al., 2022)

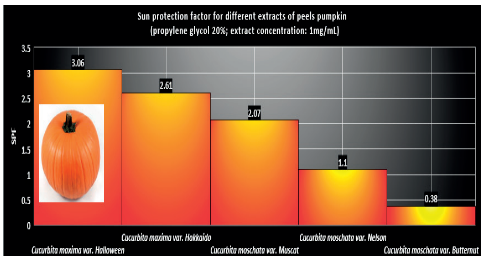


Figure 6. The sun protection factor for aqueous extracts of propylene glycol obtained from peels of different varieties of pumpkins (extract concentration = 1 mg/mL) (adapted from Gaweł-Bęben et al., 2022)

PHYTOCOMPOUNDS HIGHLIGHTED IN DIFFERENT PUMPKIN VARIETIES

Cucurbita sp. seeds contain 50% oil, primarily linoleic and oleic acid, and are rich in polyunsaturated fatty acids, oleic acid, linoleic acid, stearic acid, and myristic acid. Volatile oils are present in trace amounts in the fruit pulp of *Benincasa hispida*, commonly referred to as wax gourd or winter melon (Al-Snafi, 2013). Sugars, resins, crude fibers, free acids, carotenoids, lutein and zeaxanthin, stereos, and tocopherol are also present (Avinash and Rai, 2017).

Polyphenolic compounds are a significant class of phytocompounds. Pumpkin peels can be used to make biopreparations rich in these chemical compounds. Depending on the type of pumpkin used and the extraction solvent (aqueous solutions, ethanolic solutions, aqueous propylene glycol solutions), different extraction yields can be obtained (Figures 8-10) (Gawel-Baben et al., 2022, Radu et al., 2010).

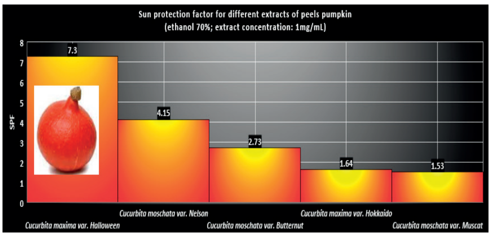


Figure 7. The sun protection factor for aqueous ethanol extracts obtained from peels of different pumpkin varieties (crude extract concentration = 1 mg/mL) (adapted from Gaweł-Bęben et al., 2022)

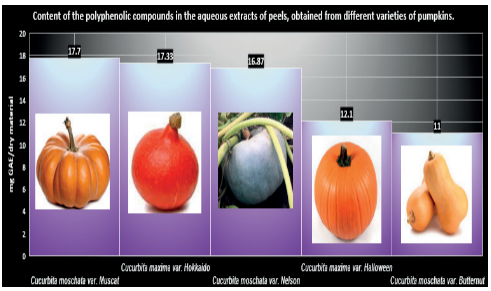


Figure 8. The content of total polyphenols in the aqueous extracts obtained from the peels of different pumpkin varieties (adapted from Gawel-Baben, 2022)

If the extraction is carried out by green methods (Sharma et al., 2021), using corn oil as an extraction solvent, and if it is assisted by ultrasound or microwaves, the content of polyphenols in the extracts obtained in the oil increases significantly. These studies report concentrations between 535-588 mg GAE/g extract, when using vegetable oil as a solvent, under action of microwaves or ultrasound (Sharma et al., 2021).

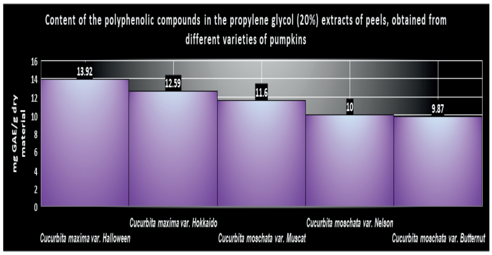


Figure 9. The content of total polyphenols in the aqueous extracts of propylene glycol obtained from the peels of different pumpkin varieties (adapted from Gawel-Baben, 2022)

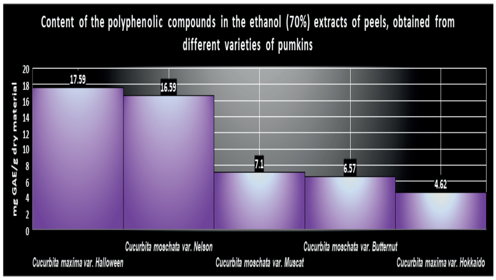


Figure 10. Content of total polyphenols in the extracts obtained from the peels of different pumpkin varieties (adapted from Gawel-Baben, 2022)

Nandhini and Sheeba (2020) highlighted the formation of silver nanomaterials using aqueous extracts from the peels of two pumpkin species (*Cucurbita* sp. and *T. cucumerina* var. *anguila*). The aqueous extract was obtained from 20 g of dried pumpkin peels, which were boiled with 100 mL of water for 10 minutes, after which the resulting solution was cooled and filtered. By treating the clear filtrate with $AgNO_3$ (10 mL of aqueous pumpkin peel extract + 90 mL of 1 mM $AgNO_3$ solution), silver nanoparticles (AgNPs) are obtained after a 20-minute maturation. After a series of distilled water washes, the newly formed nanomaterials are centrifuged at 1500 rpm, and the pellets are then kept at -4°C. Testing the antibacterial activity of the two types of nanomaterials revealed effects on all tested bacterial strains; the best results being obtained for *S. aureus* (inhibition diameter = 15.5 mm) in the case of AgNPs phytosynthesized with aqueous extract of *Tricosanthes cucumerina* (Figure 11) and respectively for *Pseudomonas aeruginosa* in the case of AgNPs phytosynthesized with aqueous extract of *Cucurbita sp.* (Figure 12). Soltani and colab. (Soltani et al., 2021) highlighted antitumor effects for AgNps obtained with ethanolic extracts from ground dry peels of *T. cucumerina*.

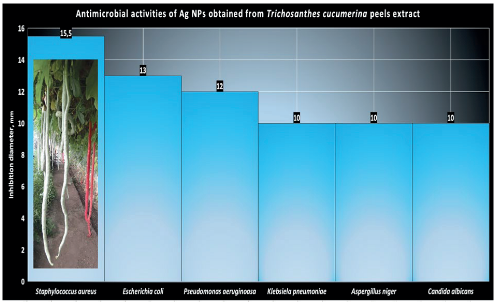


Figure 11. Antimicrobial activity of AgNPs obtained from *Trichosanthes cucumerina* var *anguina* (adapted from Nandhini & Sheeba, 2020)

The extract was obtained by mixing for 2 hours 10 grams of dried and ground pumpkin peels with 100 mL of 70% ethanol (aqueous solution). After the separation of the two phases, the alcohol is removed from the clear solution under vacuum, in a rotary evaporator, this way obtaining crude extract from pumpkin peels. If the clear alcoholic extract is treated with $AgNO_3$ (30 cm clear alcoholic extract +70 cmc $AgNO_3$ 1mM added in drops), a suspension containing AgNPs is obtained after 24 h of maturation in the dark.

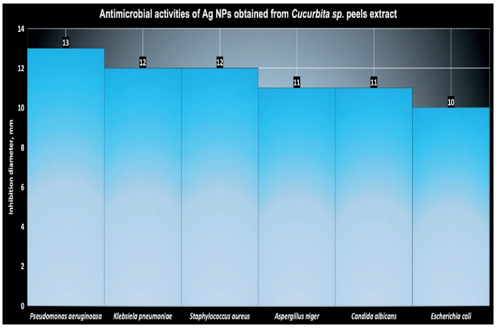
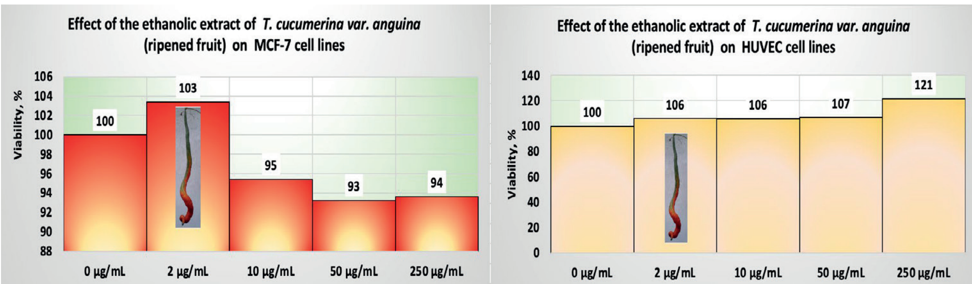


Figure 12. Antimicrobial activity of AgNPs obtained from *Cucurbita* sp. (adapted from Nandhini & Sheeba, 2020)

The obtained results showed that the crude extracts obtained from the peels of *T. cucumerina* var. anguina in 70% ethanol have cytotoxic effects both on MCF-7 tumor cells and on normal HUVEC type cells (Figure 13 a, b). In contrast, in the case of phytosynthesized nanoparticles with ethanolic extracts from *T. cucumerina* peels, optimal results are obtained for a concentration of AgNPs of 50 µg/mL, for which the tumor cell viabilities reaches 34.67% and the viability of the normal cells = 70,64%. The use of higher concentrations of AgNps results in the destruction of tumor cells in a proportion of about 82% (cytotoxicity = 82.2%) but also of normal cells, in a proportion of about 70% (cytotoxicity = 69.8%) (Figure 14 a, b).



a b

Figure 13. The antitumor activity of the ethanolic extract obtained from *T. cucumerina*:
a) the effect of the crude extract on the standardized cell line MCF-7; b) the effect of the crude extract on the standardized HUVEC cell line (adapted from Soltani et al., 2021)

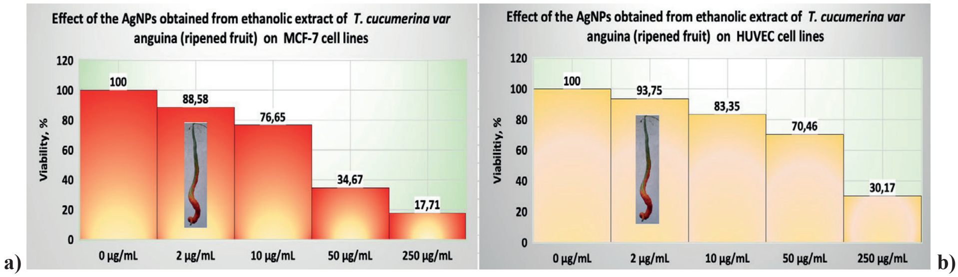
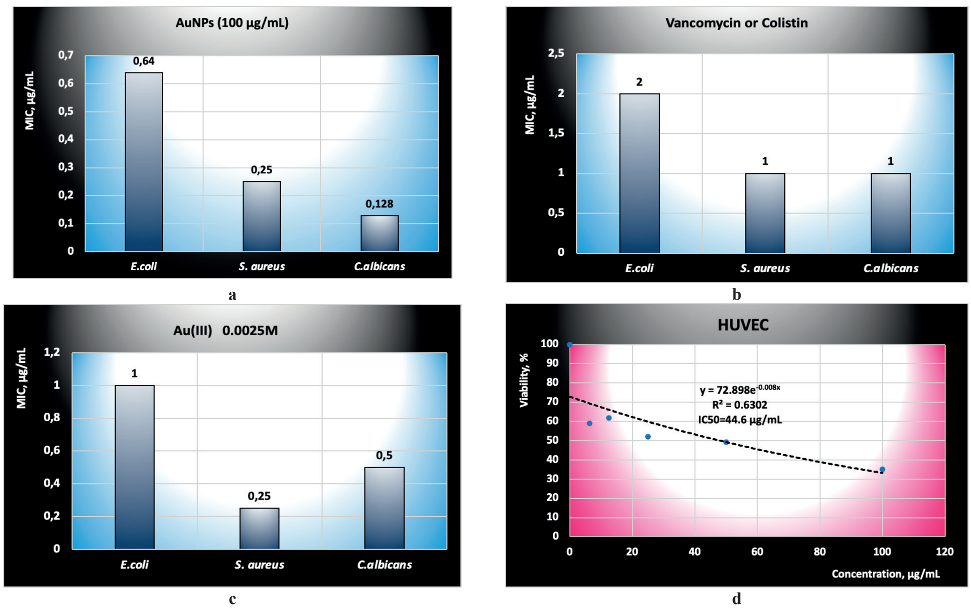
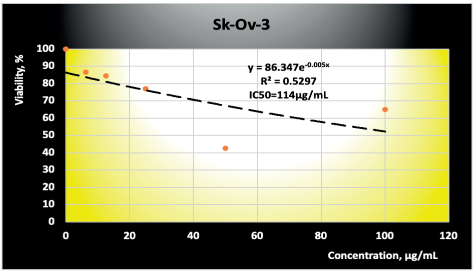


Figure 14. Antitumor activity of AgNPs obtained from T. cucumerina peels: a) the effect of AgNPs on the standardized cell line MCF-7; b) the effect of AgNPs on the HUVEC cell line (adapted from Soltani et al., 2021)

Studies performed by Kaval and colab (Kaval et al., 2024) indicate that an aqueous extract made from *Cucurbita moschata* dry peels can be used as a starting point in the production of gold nanoparticles (AuNPs). It is important to highlight that the material was ground and allowed to dry at ambient temperature. At 50°C, the extraction process was conducted using distilled water and a mass ratio of 1: 3 for the plant to solvent. After passing the solution through 45 microm membranes, the filtrate was heated for 15 minutes at t = 50°C and treated with an Au^{3+} solution (250 mL Au^{3+} 0.01M solution + 750 mL plant extract). After 15 minutes, the heating was stopped, and after about 1 hour the solution changed color from yellow to red. The resulting suspension was centrifuged at 15000 rpm and the resulting pellets were dried at 85°C, cooled, ground, and stored in sealed tubes at 4°C. In this way, nanomaterials with gold were obtained, with a size of about 21.2 nm, containing 30% Au and 56.6% C. Phytosynthesized gold nanomaterials have antimicrobial effects for *E. coli* (MIC = 0.64 µg/mL), *S. aureus* (MIC = 0.25 µg/mL) and *C. albicans* (MIC = 0128 µg/mL) (Figure 15 a-b) (Kaval et al., 2024). Cytotoxicity tests performed with these materials on normal standardized human cell lines type HUVEC and respectively on standardized human tumor cell lines Sk-Ov-3, A549, CaCo-2 showed that they are cytotoxic for the HUVEC line (IC50 = 44.6µg/mL), have average cytotoxicity for the Sk-Ov-3, A549 type tumor cell lines and do not show cytotoxicity for the CaCo-2 type tumor cell line (Figure 15 d-g) (Kaval et al., 2024; Ciric et al., 2023).





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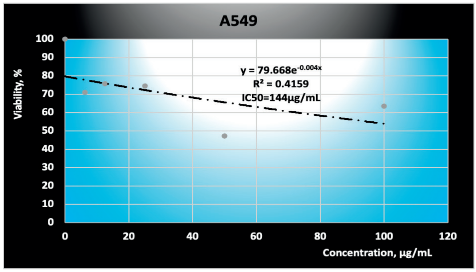

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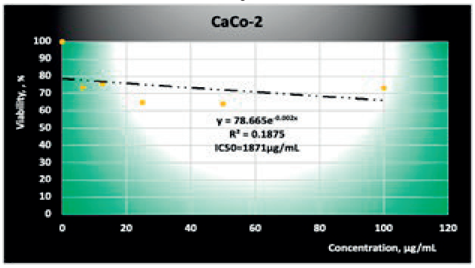

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Figure 15. Biological activities of AuNps phytosynthesized with pumpkin peel extract:
a) antimicrobian effect of AuNP;
b) antimicrobial effect of antibiotic reagent;
c) antimicrobial effect of Au^{3+} raw material;
d) antitumor effect on the HUVEC cell line;
e) antitumor effect on the Sk-Ov-3 cell line;
f) antitumor effect on the A549 cell line;
g) antitumor effect on the CaCo-2 cell line
(data adapted after Kaval et al., 2024)

Triterpenoids. One important feature of plants in the Cucurbitaceae family is the presence the triterpenoids compounds type cucurbitacins.The cucurbitacins consists of a tetracyclic cucurbitan core with a variety of oxygenated functional groups. Cucurbitacins A-T are among the twelve groups of cucurbitacins non-glycosylated or glycosylated. Different degrees of unsaturation and the presence of several keto, hydroxy, and acetoxy groups are the characteristics of cucurbitacins structural (Chen et al., 2005; Rajasree et al., 2016; Saboo et al., 2013; Montesano et al., 2018; Kaushik et al. 2018). The highest concentrations of curcubitacins are found in mature fruits; the lowest concentrations are found in seeds (Saboo et al., 2013; Kaushik et al., 2015). These compounds are typically harmful, but at the proper dosage, they exhibit beneficial qualities, particularly those related to anti-inflammatory effects, which make them helpful in the treatment of autoimmune disorders. According to Bartalis and Halaweish (2005), the degree of hydrophobicity of cucurbitacins grows linearly and is a primary regulator of their cytotoxic effects. For cucurbitacins type B and E, therapeutic properties have been highlighted and currently these compounds are used in clinical trials (Rajasree et al. 2016; Saboo et al., 2013; Montesano et al., 2018; Kaushik et al. 2018). Cucurbitacin E and its glycosidic forms are the most abundant chemical constituents in plants of the Cucurbitaceae family (Dhiman et al., 2012). Chanda and colab., (Chanda et al., 2019) used HPLC techniques to study the fruits of the following plants: *Lagenaria siceraria* (gourd), *Benincasa hispida* (wax pumpkin), *Momordica charantia* (bitter cucumber), *Coccinia grandis* (ivy gourd), and *Luffa acutangula* (cucumber sponge/loofah with edges) and found in these Cucurbitacin E. Studies conducted *in vitro* and *in vivo* have demonstrated the anti-inflammatory, anti-angiogenesis, immunomodulatory, cytotoxic, cytostatic, and hepatoprotective properties of cucurbitacins (Attard and Cuschieri, 2004). In the Table 2 are described some cucurbitacins and their biological activity.

Table 2. Types of cucurbitacins and their biological activities

Cucurbitacin	Molecular structure	Varieties	Biological properties	References
Cucurbitacin B	Glycosidic form dihydrocucurbitacin Dihydroiso-cucurbitacin isocucurbitacin B	Cucurbitaceae	Antitumor (prostate cancer, small cell lung cancer), Inflammatory, Hepatoprotective, Synergistic effect in the presence of cucurmin (activates the occurrence of apoptosis) for liver tumor	Miro, 1995; Kausar et al., 2013; Wang et al., 2014; Ma J. et al., 2014; Gao et al., 2014; Sun et al., 2015; Mukherjee et all, 2019.
Cucurbitacin D	Glycosidic form dihydrocucurbitacin D deoxycucurbitacin D epi-isocucurbitacin D	Cucurbitaceae	antiproliferative activity, cytotoxic activity, induces cell cycle arrest and apoptosis in breast carcinoma cells	Chen et al., 2005; Abbas et al., 2013; Ku et al., 2015
Cucurbitacin E	Glycosidic form dihydrocucurbitacin E dihydroisocucurbitacin E isocucurbitacin E	*Ecballium elaterium*, *Coccinia grandis*	Neuroprotective, anti-inflammatory, antipyretic, anti-tumor (breast cancer), anti-allergic, anthelmintic, purgative activity	Miro, 1995; Yoshikawa et al., 2007; Lan et al., 2013; Abbas et al., 2013; Arel-Dubeau et al., 2014; Abdelkhalek et al., 2017; Lu et al., 2017; Chanda et al., 2020
Cucurbitacin F	dihydrocucrbitacin F, hexanorcucurbitacin F, oxocucurbitacin F și forma glucozidică; 23, Dihydro și 15-oxo cucurbitacin F (3), 15-oxo-23, 24-dihydro cucurbitacin F		used in traditional Chinese medicine, anti-HIV activity	Konoshima et al., 1994; Chen et al., 2005
Cucurbitacin I			cytotoxic activity, antitumor activity, chemotherapy adjuvant agent	Abbas et al., 2013; Johnson et al., 2013
Khekadaengosides D, Deoxycucrbitacin glycoside (Spinosides A)	(Spinosides A)		antitumor activity	Mukherjee și colab., 2022
Cucurbitacin L, or J or K	brydioside A bryoamaride		No therapeutic activity	Mukherjee și colab., 2022

Carotenoids. Most plants from Cucurbitaceae family are rich in carotenoids. Over 700 carotenoids occur naturally, but only 50 can be absorbed, metabolized, and used by the human body with health benefits. In Cucurbitaceae, they occur as a-carotene, β-carotene, lutein, zeaxanthin (Montesano et al., 2018; Durante et al., 2014). The total carotenoid content of *Cucurbita moschata* varies from 234.21 to 404.98 mg/g dried fruit. The carotenoid content is often higher in the peel than in the pulp. For example, in *Cucurbita moschata* the carotenoid content is 10 times higher in the peel than in the pulp (Salehi et al., 2019). In the peels of several varieties of pumpkins derived from *C. maxima* and *C. moschata*, grown in Umbria Italy, Pinna and colab. (Pinna et al., 2023) highlighted the existence of compounds such as beta carotene (Figure 16), non-esterified carotenoid compounds (Figure 17) such as α-carotene, β-carotene, lutein, zeaxanthin, violaxanthin and esterified carotenoids compounds (Figure 18) (Bunea et al., 2014). The studies carried out by Sharma et al. (Sharma et al., 2021) on two varieties of *Cucurbita maxima*, demonstrated that the extraction yield of carotenoid compounds from peels can increase by 100% when using green extraction methods (Figure 19), compared to the variant in which extraction is carried out with organic solvent. The concentration of carotenoids was estimated spectrophotometrically, with the relation (1) (Sharma et al., 2021):

$$C = \frac{\text{Ax1000000}}{\text{2000x100xd}} \qquad (1)$$

C = mg/Kg oil; D = thickness of the tub (usually 1 cm)

In the green extractions methods studied by Sharma et al. (Sharma et al., 2021) corn oil was used as the extraction agent and the extraction process was assisted by microwaves or ultrasound. In this way, the extraction yield of polyphenolic compounds can increase by 200%, and the color and oxidation stability of biopreparations enriched in carotenoid compounds is greatly improved (Figure 20 a, b). These processes are particularly recommended for specific applications in the food or cosmetic industry.

The studies performed by Gaweł-Bęben and colab. (Gaweł-Bęben et al., 2022) demonstrated that active principles from crude extracts otained from the peels of various varieties of pumpkin (*Cucurbita* spp.) could be used directly in dermato-cosmetic bioproduct formulation.

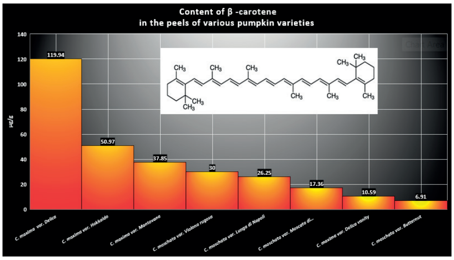


Figure 16. The content of beta β carotene in peels from different varieties of *Cucurbita* sp. (adapted from Pinna et al., 2023; Bunea et al., 2014)

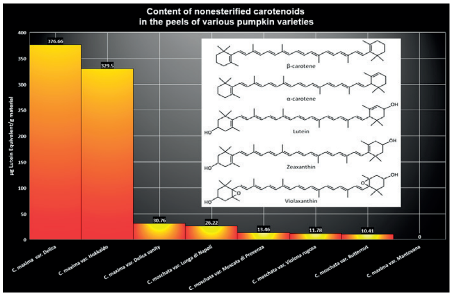


Figure 17. The content of non-esterified carotenoids in peels from different varieties of *Cucurbita* sp. (adapted from Pinna et al., 2023; Bunea et al., 2014)

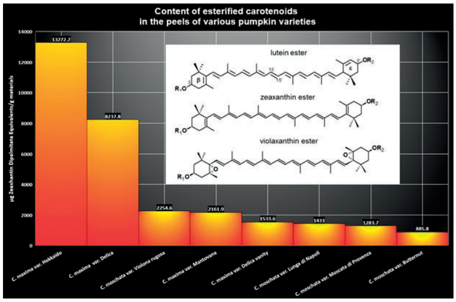


Figure 18. The content of non-esterified carotenoids in peels from different varieties of *Cucurbita* sp. (adapted from Pinna et al., 2023; Bunea et al., 2014)

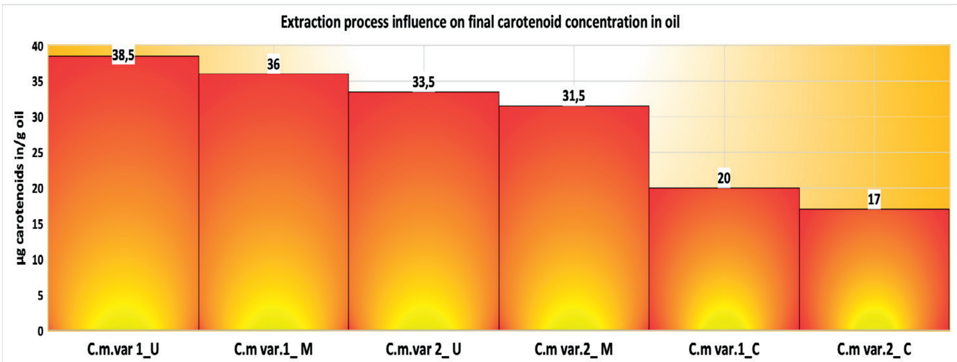
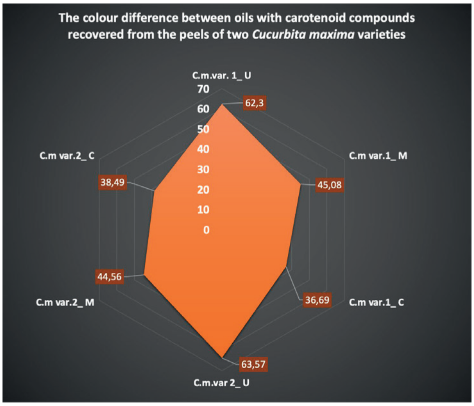


Figure 19. The effect of the extraction process on the content of carotenoid compounds in the final solvent (corn oil) (adapted from Sharma et al., 2021)

(Legend: C.m. var.1_U= carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction in corn oil, assisted by ultrasound; C.m.var.1_M = carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction in corn oil, assisted by microwaves; c.m. var.1_C=carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction with organic solvents; c.m. var.2_U=carotenoids obtained from peels of *C. maxima* var. Amoro, by extraction in corn oil, assisted by) ultrasound; c.m. var.2_M= carotenoids obtained from peels of *C. maxima* var. Amoro, by extraction in corn oil, assisted by microwaves; c.m. var.2_C= carotenoids obtained from peels of *C. maxima* var. Amoro, by extraction with organic solvents)



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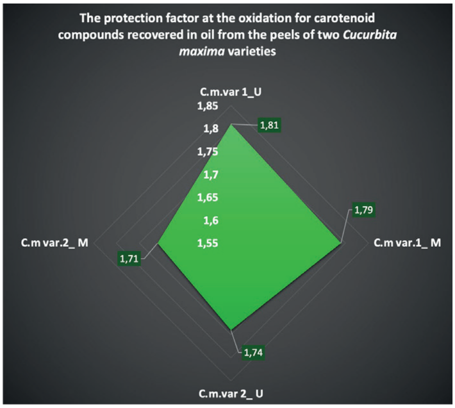

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Figure 20. The effect of the extraction process, on the color difference (ΔE) and stability at oixidation of bioproducts obtained in corn oil (adapted from Sharma et al., 2021)

(Legend: C.m. var.1_U= carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction in corn oil, assisted by ultrasound; C.m.var.1_M= carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction in corn oil, assisted by microwaves; c.m. var.1_C=carotenoids obtained from peels of *C. maxima* var. Gold nugget, by extraction with organic solvents; c.m. var.2_U=carotenoids obtained from peels of *C. maxima* var. Amoro, by extraction in corn oil, assisted by ultrasound; c.m. var.2_M=carotenoids obtained from peels of *C. maxima* var. Amoro, by extraction in corn oil, assisted by microwaves; c.m. var.2_C= carotenoids obtained from peels of *C. maxima* var.Amoro, by extraction with organic solvents)

CONCLUSIONS

The by-products resulting from the processing of Cucurbitaceae fruits (such as the peels) represent a valuable source of raw materials, due to their content in alkaloids, polyphenolic compounds, terpenoids, saponins or carotenoid compounds.

In general, by extraction in aqueous medium or by extraction with organic solvents such as alcohols (ethanol, methanol), acetone, n-hexane, water, propylene glycol, from fresh or dry peels, bioproducts with an antibacterial (*S. aureus* and *E. coli*) , and antifungal effect (*C. albicans*, *Fusarium solani*, *P. chrysogenum*, *C. gloeosporioides* - anthracnose) can be obtained.

Extracts in ethanol or propylene glycol may contain carotenoid compounds, but the most advantageous methods of extracting them from cucurbit peels are green methods, in which are used as solvents vegetable oils, and the extraction process is intensified by microwaves or ultrasounds, In this case the bioproducts obtained may contain simple carotenoids of type α or β carotene, lutein, zeaxanthin, violaxanthin, or esterified carotenoids. The bioproducts obtained by green extraction methods have a sun protection factors between (1-7) and can be used directly in the cosmetic formulations.

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CHANGES IN MICROBIAL ABUNDANCE OF COMPOST AFTER TREATMENT WITH SPECIFIC ADDITIVES

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Abstract

The present study aimed to follow the change in the microbial community of finished compost after application of different additives. Six variants and one control (C) were prepared: V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - mineral + microbial fertilizer. Initial sampling was performed, as well as sampling at days 5, 10, and 15. In V2 V5, V6 and V7, the microbial biota increases with time. On the fifth day, the amounts of microorganisms were highest in the samples with added organic aqueous extracts. On the tenth day, the V7 has the highest microbial abundance. On the fifteenth day, the microbial abundance of V5 is again the lowest. The highest number of microorganisms has V7. The data showed that the water-based organic extracts slightly increased the amount of the microbial community compared to the control. The pure microbial additive has a suppressive effect on the compost microflora, but combined with a mineral additive gives good results and can be used for improvement of compost parameters.

Key words: *microorganisms, compost, composting process, additives.*

INTRODUCTION

Composting represents an economically advantageous methodology for biological decomposition. The regulation of the composting process is predominantly governed by microorganisms. This intricate process is significantly influenced by the physicochemical parameters, including thermal conditions, oxygenation levels, moisture content, carbon to nitrogen ratio, and pH (Cogger et al., 2008; Ventorino et al., 2019).

FAO defines composting as a specific aerobic process in which aerobically composted organic matter is converted into a mass called "compost". This mass is used to improve soil structure and provide nutrients (FAO, 2015). During the composting process, microorganisms decompose organic material, resulting in the generation of carbon dioxide, water, heat, and humus - the comparatively stable organic resultant product. Under conditions deemed optimal, the composting sequence progresses through four distinct phases: 1) the mesophilic or moderate-temperature phase; 2) the thermophilic or elevated-temperature phase; 3) a cooling phase and 4) maturation phase, spanning several months (Papale et al., 2021). Specific groups of microorganisms are involved in all phases of the composting process. The products obtained as a result of metabolic processes of one type of microorganisms serve as nutrients or a source of energy for another type of microorganisms (Mira et al., 2003). The main participants in the composting process are bacteria, microscopic fungi and actinomycetes. Bacteria play the main role in the individual stages of composting (Silva & Naik, 2007).

Composting as a process is a hot topic in agriculture. Composting achieves simultaneous treatment and disposal of the generated agricultural waste and subsequent production of a valuable product - compost (Wagas et al., 2023; Papale et al., 2021).

Across Europe, agricultural soils are degraded and in poor condition both in terms of their physical performance and nutrient content (Ferreira et al., 2022; Lawrence & Melgar, 2023). Composting is an economically beneficial process that can help both small farmers and medium and large farmers to help restore the soil. (Karthika, et al., 2022). When applying compost to the soil, a number of improvements in its characteristics are observed - the structure of the soil is improved, the water-air regime is improved, the pH is balanced and slow-release nutrients are introduced (Lucchetta et al., 2023; Ahmed et al., 2023). Compost is an increasingly preferred product over mineral fertilizers, because when it is applied, the nutrient elements are absorbed slowly, which minimizes the possibility of competition between soil microorganisms and the cultivated crop (Maucieri et al., 2019). In addition, when compost is applied, it is not washed away in depth, as is the case with inorganic fertilizers. Composting is a promising environmentally friendly process for dealing with organic waste generated by agriculture (Sayara et al., 2020). The introduction of specific additives to the finished compost can improve its qualities, both from a sanitary point of view and from a microbiological point of view (Barthod et al., 2018).

The **main** objective of the present study is to follow the change in the microbial community of compost after the application of different additives in it.

MATERIALS AND METHODS

For the purpose of the present study, a compost obtained by mixing sprouted potatoes: wood chips: lettuce: soil in a ratio of 20:2.5:10:7.5 was used. The ratio of carbon to nitrogen in the starting mixture is 31:1.

Six variants and one control (C) were prepared.

The extract variants were prepared according to the Decoction method: The extracts were prepared from whole plants of the essential oil crops used (lavender, thyme, basil ets.) by boiling the crushed plant material from the respective plant in solvent water and subsequent hot filtration. The analyzed variants are as follows:

V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix of lavender, thyme and basil extract, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - mineral + microbial fertilizer. Basic microbiological indicators were investigated immediately after preparation of the mixes and the control, as well as after 5, 10 and 15 days.

Basic microbiological indicators include the determination of non-spore-forming bacteria, spore-forming bacteria (such as *Bacillus* sp., etc.) *Lactobacillus* spp., actinomycetes (*Saccharomonospora* spp., *Streptomyces* spp.) and microscopic fungi (*Aspergillus* sp, *Penicillium* sp. etc.). The analysis for the number of microorganisms was performed by microbiological groups by inoculation of (semi)selective agar media. No separate identification of species from the groups of non-spore-forming bacteria, spore-forming bacteria, actinomycetes and microscopic fungi was carried out. Based on the obtained data, the total microbial count of each of the samples was calculated. For microbiological analyses, the standard Koch method was used, including serial dilutions and using plate counts in CFU on (semi)selective agar media. Water extracts of lavender (V1), thyme (V2), basil (V3), a mixture of the three (V4), microbial fertilizer (V5), mineral fertilizer (V6) and a mixture of microbial and mineral fertilizer (V7) were used to prepare the different variants.

RESULTS AND DISCUSSIONS

Table 1. presents the results of the performed microbiological analyses. The results show an increase in microbial abundance compared to the initial phase in all variants, including the control.

In the starting initial compost, non-spore-forming bacteria represented 65% of the microbial community, followed by spore-forming bacteria at 16%. With almost equal amounts in the microbial community are micromycetes and actinomycetes, respectively 9% and 8%. The participation of lactobacilli is the least (2%).

Total microbial results vary by variant and time of reporting (Figure 1.)

The results show that the introduction of additives affects the total microbial count of the finished compost. All variants showed an increase in the total amount of microorganisms on the fifth day compared to the initial compost (Figure 1).

Table 1. Main microbiological parameters (CFU*10^6/g compost)

	TMN	Non-spore-forming bacteria	Spore-forming bacteria	*Lactobacillus* spp.	Actinomycetes	Micromycetes
0	1.18	2.15	0.54	0.07	0.27	0.30
5th day						
K	5.0 4 ± 0.80	3.18 ± 0.89	0.94 ± 0.07	0.07 ± 0.10	0.81 ± 0.30	0.05 ± 0.06
1	5.36 ± 0.80	3.28 ± 0.89	1.04 ± 0.07	0.07 ± 0.10	0.87 ± 0.30	0.10 ± 0.06
2	5.36 ± 0.80	3.19 ± 0.89	0.96 ± 0.07	0.34 ± 0.10	0.77 ± 0.30	0.10 ± 0.06
3	5.59 ± 0.80	3.19 ± 0.89	1.01 ± 0.07	0.08 ± 0.10	1.18 ± 0.30	0.13 ± 0.06
4	5.22 ± 0.80	3.18 ± 0.89	0.89 ± 0.07	0.02 ± 0.10	1.01 ± 0.30	0.13 ± 0.06
5	4.45 ± 0.80	1.44 ± 0.89	1.06 ± 0.07	0.03 ± 0.10	1.68 ± 0.30	0.24 ± 0.06
6	3.36 ± 0.80	1.38 ± 0.89	0.87 ± 0.07	0.13 ± 0.10	0.87 ± 0.30	0.10 ± 0.06
7	3.90 ± 0.80	1.66 ± 0.89	1.01 ± 0.07	0.08 ± 0.10	1.06 ± 0.30	0.08 ± 0.06
10th day						
K	4.84 ± 0.46	2.52 ± 0.43	0.97 ± 0.06	0.10 ± 0.13	1.18 ± 0.25	0.07 ± 0.02
1	5.34 ± 0.46	3.29 ± 0.43	1.02 ± 0.06	0.08 ± 0.13	0.87 ± 0.25	0.07 ± 0.02
2	5.71 ± 0.46	3.33 ± 0.43	0.97 ± 0.06	0.45 ± 0.13	0.84 ± 0.25	0.12 ± 0.02
3	4.99 ± 0.46	3.28 ± 0.43	0.96 ± 0.06	0.10 ± 0.13	0.59 ± 0.25	0.07 ± 0.02
4	5.48 ± 0.46	3.24 ± 0.43	0.91 ± 0.06	0.02 ± 0.13	1.21 ± 0.25	0.10 ± 0.02
5	4.54 ± 0.46	2.18 ± 0.43	1.04 ± 0.06	0.05 ± 0.13	1.18 ± 0.25	0.08 ± 0.02
6	5.06 ± 0.46	3.02 ± 0.43	0.89 ± 0.06	0.17 ± 0.13	0.87 ± 0.25	0.10 ± 0.02
7	5.93 ± 0.46	3.31 ± 0.43	1.04 ± 0.06	0.10 ± 0.13	1.34 ± 0.25	0.13 ± 0.02
15th day						
K	4.91 ± 0.50	2.55 ± 0.43	0.99 ± 0.06	0.10 ± 0.13	1.18 ± 0.23	0.08 ± 0.03
1	5.66 ± 0.50	3.38 ± 0.43	1.08 ± 0.06	0.10 ± 0.13	1.01 ± 0.23	0.10 ± 0.03
2	5.93 ± 0.50	3.36 ± 0.43	1.01 ± 0.06	0.45 ± 0.13	0.97 ± 0.23	0.13 ± 0.03
3	5.56 ± 0.50	3.36 ± 0.43	1.02 ± 0.06	0.13 ± 0.13	0.94 ± 0.23	0.10 ± 0.03
4	5.06 ± 0.50	3.29 ± 0.43	0.94 ± 0.06	0.03 ± 0.13	0.67 ± 0.23	0.12 ± 0.03
5	4.79 ± 0.50	2.27 ± 0.43	1.08 ± 0.06	0.08 ± 0.13	1.24 ± 0.23	0.12 ± 0.03
6	5.38 ± 0.50	3.16 ± 0.43	0.92 ± 0.06	0.20 ± 0.13	0.97 ± 0.23	0.12 ± 0.03
7	6.20 ± 0.50	3.39 ± 0.43	1.08 ± 0.06	0.13 ± 0.13	1.43 ± 0.23	0.17 ± 0.03

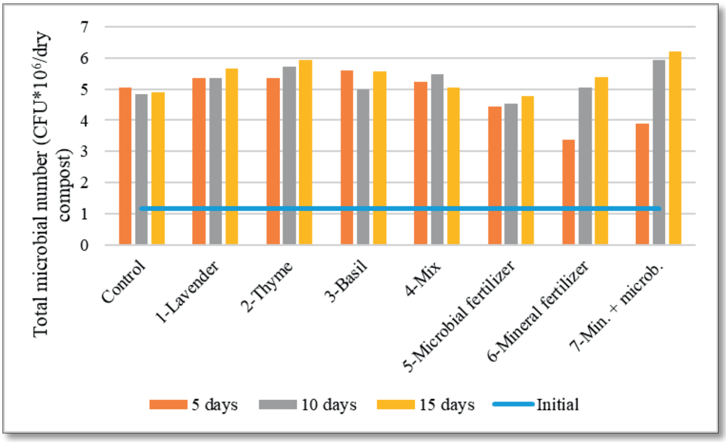

Figure 1. Total microbial number (CFU*10^6/g compost)

All variants with added plant extracts showed higher biogenicity than the control. With the lowest microbial abundance on the fifth day are the variants with added microbial supplement, mineral supplement and a mix of both: V5=4.45*10^6 CFU/g compost mass, V6=3.36*10^6 CFU/g compost mass, V7=3.90*10^6 CFU/g compost mass.
The highest biogenicity on the fifth day was reported for sample three - with added water extract from basil.
The results on the tenth day showed an increase in the microbial biota in sample 4 and 5. An increase in microbial abundance was also observed in the thyme variants (Sample 3). However, the greatest increase in microbial biota was observed in sample 6 and sample 7 (Figure 1). These are the samples with delayed microbial community development on the fifth day compared to the initial compost.
On day ten, variants 6 and 7 reached an approximate value as the others, but on day 15 variant seven had the highest total microbial count. These data show that a combination of microbial fertilizer and mineral fertilizer at the beginning of enrichment on day 5 does not stimulate the microbial biota, but after that the data show a positive effect on the amount of microorganisms.
On the fifteenth day, we have an increase in the amount of microorganisms in all the examined samples compared to the tenth day, with the exception of the sample with an added mix of plant water extracts. Of the tested mixtures, only the mixture of water extracts showed a decrease in the microflora on day 15. The control data is the most passive. After the rise on day 5 we have a slight decline on day 10 and a slight rise again on day 15.
The information generated by the present study shows that the application of the mineral fertilizer and the complex of mineral fertilizer and microbiological additive to the finished compost increases the microbial potential of the compost, due to the heights microbial number of V7 at day 15.
Lavender in principle shows antimicrobial activity against pathogenic and non-pathogenic microorganisms (Kwiatkowski et al, 2020), therefore we assume that it has a restraining effect on the microbial biota, in the initial days of the experiment, as there is almost no increase in total microbial numbers.
Of interest is Variant 5, which is only with added microbial fertilizer. There, a retention of microbial abundance was observed, with levels lower than those of the control during all three samplings. Such data show the presence of competition between the autochthonous microbiota of the compost and the imported allochthonous microbiota with the microbial fertilizer. In other soil-related studies when allochthonous microorganisms were introduced, the autochthonous did not struggle and outcompeted it (Podmirseg et al., 2019).
These results show the higher sensitivity of the local microorganisms in the compost compared to introduced external microorganisms. The present study found a suppressive effect of the imported microbial supplement in all three consecutive samplings. In all the analyzes performed, the sample with only the microbial additive introduced showed lower biogenicity compared to the control.
Figure 2 presents results for the percentage participation of individual microbiological groups.
In the initial compost, the group of non-spore-forming bacteria was the most involved, followed by bacilli. Actinomycetes and micromycetes have an almost equal share. Lactobacilli have the smallest share of less than 3%. Through all three subsequent sampling and analysis of the compost variants, the data were almost identical.
The participation of actinomycetes increased, at the expense of non-sporulating bacteria and micromycetes. This trend persists until day 15.
In almost all variants except the variant with microbial fertilizer, the group of non-spore-forming bacteria dominates. An exception is the sample with added microbial fertilizer.
The predominance of non-spore-forming bacteria is typical because these microorganisms are involved in the cooling phase of the compost as well as during the maturatione phase (Varma et al., 2018).

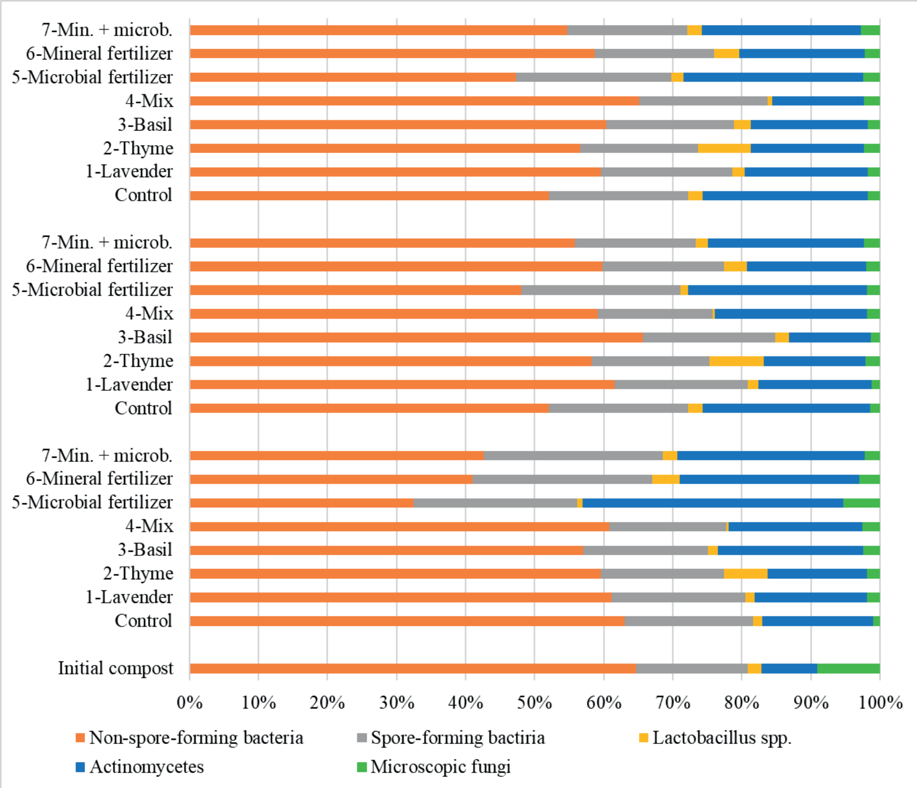

Figure 2. Percentage participation of individual microbial groups

The large amount of bacteria in general relative to the total biogenicity of compost is related to the ability of these microorganisms to adapt to different environmental conditions much faster than other microbial groups (Shi et al., 2022; Aguilar-Paredes et al., 2023). Bacteria are the main microorganisms carrying out the composting process, as they are adapted to the consumption and decomposition of various organic substances (Golueke, 1992; Epstein, 1997).

With the smallest percentage share are *Lactobacillus* spp., given their increased participation in the early stages of the composting process (Kurola et al., 2011; Tran et al., 2019)

When considering the percentage participation of the individual functional groups of microorganisms in relation to the totality of the microbial community, certain trends are clearly visible. From the obtained results it is evident that the microbial additive suppresses the development of the more dominant in the compost, a group of non-compost-forming bacteria.

Of all the investigated variants, the sample with the added microbial supplement showed the greatest reduction in the percentage of non-spore-forming bacteria. On the fifth day after the introduction of microbiological fertilizer, the percentage of non-spore-forming bacteria drops to approx. 30% due to the increased participation of actinomycetes with nearly 40% participation.

A decrease in the percentage of lactobacilli was observed in the variant with a mix of plant extracts. Based on these results, we do not recommend the use of a mixture of the investigated extracts to improve the compost qualities, since lactobacilli, even with a small percentage, play an extremely important role. *Lactobacillus* spp. are associated with stopping unpleasant odors during composting. Lactobacilli use ammonia as a food source, which is released during decomposition and can lead to the release of an unpleasant odor (Varma et al., 2018).

Given the results generated, when unpleasant odors are present in the compost, the addition of thyme extracts will increase the percentage of Lactobacilli and regulate the depletion of generated ammonia.

We considered that the imported additives, with the exception of the microbial extract, as well as the variant with aqueous thyme extract, do not have a significant impact on the distribution of the percentage participation of the microbial groups.

CONCLUSIONS

Seven variants of compost mixes and one control were studied. The results show that the applying of additives affects the total microbial number of the finished compost. The present study found a suppressive effect of the added microbial supplement in all three consecutive samplings. In all the analyzes performed, the sample with an imported microbial supplement showed lower biogenicity compared to the control.

However, when using a combination of microbial supplement and mineral fertilizer, the highest levels of microbial abundance were generated relative to the control on day 15.

In all variants with added aqueous extract, an increase in the total microbial number was observed compared to the control.

The obtained results for the percentage distribution of the microbial groups, again show that the used microbial additive is not recommended as an improver of the qualities of the finished compost.

In this sample, the amount of non-spore-forming bacteria decreases sharply, at the expense of actinomycetes. Also, the microbial fertilizer reduces the percentage of lactobacilli.

The present study found that of the plant extracts, the addition of thyme extract was the most suitable option for improving the qualities of the finished compost. When adding an aqueous extract of thyme, a general increase in the number of microorganisms was observed, as well as an increased percentage of Lactobacilli.

We also consider the combination of microbial fertilizer and mineral fertilizer as a suitable supplement, but after standing the mixture for fifteen days.

The present study can serve as a basis for future studies related to the influence of specific additives introduced into finished compost.

ACKNOWLEDGEMENTS

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USING CYTOKININ TO ENHANCE ESSENTIAL OIL BIOSYNTHESIS OF TWO ROSE GERANIUM (*PELARGONIUM GRAVEOLENS* L.) VARIETIES: REUNION AND MADAGASCAR-TYPE

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Abstract

Previous studies have demonstrated that exogenous application of phytohormone-based biostimulants and subjecting aromatic plants to environmental stresses improves the biosynthesis of secondary metabolites. This experiment was laid out in a 5×2 factorial design, arranged in a randomised complete block design, and replicated three times to determine the effects of cytokinin (CK) on essential oil biosynthesis. Treatments consisted of four CK (221.6, 443.2, 664.8, 886.4 µM and control); and two varieties of rose geranium (Pelargonium graveolens L.): Reunion-type, and Madagascar-type. Analysis of variance and multivariate analysis showed that CK between 221.6 and 664.8 µM may improve the essential oil biosynthesis for the two varieties. Linalool and geranyl tiglate were increased by CK-886.4 µM following simulated wounding on the Bourbon-type, and Madagascar-type, respectively. This study demonstrated that the Bourbon type remains better than the Madagascar type. The author concludes that the application of CK-664.8 µM closer to harvest may improve the Rhodinol and essential oil biosynthesis of both varieties.

Key words*: Biostimulant, citronellol to geraniol ratio, essential oil quality, phytohormone, Rhodinol.*

INTRODUCTION

The global essential oil market is projected to expand between 8.4% and 11.3% on an annual basis until 2025, accumulating an amount of $14.0 and $15.8 billion, ranging from 500 – 750 tons annually (Kumar et al., 2022; CBI, 2018). In recent years, production of high-quality rose geranium oil in Reunion Island has been immensely declining, thereby affording opportunities to emerging producers globally. South Africa produces rose geranium oil like that of Reunion Island, and the country has increased its rose geranium oil production by 25 tons in the past five years, making it 5 tons annually (CBI, 2018). Despite the increase, South Africa, as the origin country of the crop, could potentially contribute 50 tons of geranium oil annually to the global essential oil market (Araya, 2012). However, to optimise this espoused production yield and essential oil quality, the research to enhance and optimise the essential oil yield and quality of rose geranium is crucial.

Rose geranium is a native aromatic crop to South Africa and is globally cultivated for its leaf-extractable high-value essential oil, which is utilized in the aroma and fragrance industry, a billion-dollar market (CBI, 2018). The rose geranium essential oil comprises a complex mixture of more than 120 volatile organic compounds (VOC's) (Demarne and Van der Walt, 1989; Araya, 2012), of which few are key ingredients in cosmetics, perfumery, agrochemicals, pharmaceuticals, and aromatherapy; namely citronellol, geraniol, linalool, citronellyl formate, geranyl formate, guaia-6, 9-diene and rose-oxide (Sedibe and Allemann, 2012). These compounds occur in various proportions depending on the country of origin. The Reunion-type oil from the Island of Reunion (Reunion-type includes Reunion Island origin), for instance, is the only geranium oil with the citronellol to geraniol (C:G) ratio close to 1:1 and is seen as endowed with the highest quality (Khetsha and Sedibe, 2015). On the other hand, the C:G ratio for Madagascar type varies from 1.3 to 1.8 (ISO 4731E, 2012).

Cultivation in South Africa is dominated by populated rural areas, which are poverty-stricken (DAFF, 2012). Most of these communities depend on farming for survival, mainly cultivating rose geranium due to its demand, drought tolerance and low risk (Eiasu et al., 2022). However, a usual setback in the cultivation of rose geranium is the quantitative and qualitative difference at harvest. This results in decreased oil market value and uncertainty in the agri-businesses and a loss of opportunity afforded to emerging farmers by the market suppliers' inability to meet the continuous global demand growth for rose geranium oil.

According to Eiasu et al. (2022), Zigene and Kassahun (2023), and Khetsha et al. (2022), the low oil quality of rose geranium at harvest owes to the reduced content of essential oil compounds, at most leading to high C:G ratio, which is greatly affected by the age of plant shoots, the different environmental factors. Geraniol and linalool levels have been found to decline by 40-70% in older leaves, while citronellol and its esters remain at constant levels (Gomes et al., 2005). In addition, Eiasu et al., (2022) and Motsa et al. (2006) further corroborated that shorter growth cycles may increase essential oil biosynthesis, depending on the harvesting stage and techniques as reported by Gebremeskel (2014) and Malatova et al. (2011). Typically, hot sunny days, with average temperatures between 10 and 22ºC are suitable for high yield and essential oil biosynthesis (Zigene and Kassahun, 2023; Rao et al., 2001). In addition, since it was reported that, the essential oils are involved in plants' response to abiotic stresses (Aqeel et al., 2023); therefore, exposing aromatic plants to water stress and wounding has also been key in improving secondary metabolites accumulation (Khetsha et al., 2022; Thakur et al., 2019; Eiasu et al., 2012). Therefore, to improve the essential oil yield and quality of rose geranium at harvest, researching innovative production techniques is important.

Phytohormone-based biostimulants, such as cytokinins (CK), have been proven to control entire plant life processes, even under unfavourable growing conditions (Asghar et al., 2023; Svolacchia and Sabatini, 2023; Abualia et al., 2023). For this reason, they have been used to improve the essential oils' accumulation (Prins et al. 2010).

Norouzi et al. (2021), Khetsha et al. (2021), Thakur and Kumar (2020) and Pal et al. (2016) reported that foliar application phytohormones could influence the essential oil biosynthesis of aromatic plants, such as thyme (*Thymus vulgaris* L.), rose geranium, and damask rose (*Rosa damascena* Mill.). In addition, foliar application of CK (up to 1.18 mM) has also been shown to improve secondary metabolites and yield parameters of tobacco (*Nicotiana tabacum* L.), peppermint (*Mentha piperita* L.), and marigold (*Calendula officinalis* L.) (Santoro et al., 2013; Machado et al., 2014; Niakan and Ahmadi, 2014). Fraternale et al. (2003) in the review of Prins et al. (2010) reported an increase in the essential oil yield of *Thimus mastichina* (L.) after spraying with benziladenine (BA).

Phytohormone-based biostimulants are derived from phytohormones, and produce signalling molecules that, either alone or combined, play a key role in regulating all aspects of plant growth and development, as well as in mediating plants' responses to biotic and abiotic stresses (Singh et al., 2022; Xu et al., 2018). These compounds act in small concentrations at or near the site of synthesis and distant tissues. Their actions affect processes of signal transduction, a relay through which intracellular or extracellular signals convert into plant responses, such as essential oil biosynthesis (Khetsha et al., 2022; Wang and Irving, 2011; Prins et al., 2010). However, the responses of plants to the foliar application of these compounds depend on the species, variety, environmental conditions, developmental stage, physiological and nutritional status, hormone concentration as well as type of phytohormone and time of application (Sharafzadeh and Zare, 2011). According to Prins et al. (2010), plants produce essential oils in response to physiological stress, pathogen attack, and other ecological factors, and CK is involved in the biosynthesis of essential oil compounds. Therefore, by simulating wounding, limiting moisture, and administering CK towards harvest in this study, it is hypothesised that CK at high concentration in simulated wounding and limited moisture towards harvest may enhance the essential oil biosynthesis of the two rose geranium varieties, Reunion-type, and Madagascar-type cv.

MATERIALS AND METHODS

Experimental site and description. This experiment was carried out under a 72 m^2 plastic-covered greenhouse tunnel located at Central University of Technology, Free State, Bloemfontein. Bloemfontein (29°07'16.78''S 26°12'45.95''E) is a semi-arid area situated in the Free State province at an altitude of 1380 m above sea level. An air extraction fan system linked to a thermometer adapter was set at 26°C to regulate the day temperature in the tunnel; however, at night the temperature was not regulated.

Growing system and planting culture. Stem-propagated cuttings of rose geranium varieties, Reunion-type, and Madagascar-type with a height of ±15 cm and a diameter of 0.75-1.0 cm, were sourced from Agricultural Research Council (Tropical and Subtropical Crops, RSA), and Siyakholwa Developmental Foundation Nursery (Eastern Cape, RSA), respectively. The two varieties of stem-propagated cuttings were transplanted on the 1st of November 2018.

Experimental design. The experiment was laid out in a 5×2 factorial design, arranged in a randomised complete block design, and replicated three times. Treatments consisted of four CK (221.6, 443.2, 664.8 and 886.4 µM) and the control (distilled water); and two varieties: Reunion-type, and Madagascar-type. Each treatment unit consists of 10 plants, and each type of treatment consists of five plants.

Treatments. A nine-month experiment was conducted from November 2018 to August 2019 to determine the effects of CK (Kinetin, Sigma-Aldrich, RSA) on two rose geranium varieties. The same transplanted plant material was harvested for three growing cycles, on the 31st of January 2019, the 30th of April 2019, and the 1st of August 2019. For each growing cycle, CK treatment was administered on the same plants throughout the three harvesting cycles. In all harvesting cycles, plants were treated with CK daily, for seven days before the beginning of each harvest.

A protective dividing plastic cover was used to divide the experimental units (Figure 1), as well as a mini-plastic tunnel was utilized as a protective cover during the application in all treatments to avoid spillage and drifting over other treatments. All treatments were exogenous in the morning between 6:00 and 7:00 am to avoid the deterioration of the phytohormonal molecules (Melgoza et al., 2014). A nozzle-calibrated precision sprayer (0.3 MPa pressure) will be utilized to spray the combination treatment solutions at 20 mL/plant (Franks and Faquhar, 2001; Bano and Yasmeen, 2010).

Foliar application of CK was achieved by preparing a 1 mg/mL stock solution CK, and then adding 100 mg of the CK in a 100 mL volumetric flask. Between 3-5 mL of solvent was prepared and added to the volumetric flask to dissolve the CK powder. Once completely dissolved, it was added to a volume with distilled/deionized water. The solution was stirred while adding water to keep the material in solution. The stock solution was stored in a cool environment to avoid deterioration.

Figure 1. The demonstration of the rose geranium treatment combination divisions between treatments

During the first harvest, intact rose geranium plants were harvested by mechanically cutting off the stem using a pair of handheld secateurs at about ±15 cm from the ground, specifically on the last seventh day after CK treatment. Plants were allowed to regrow for three more months.

For the second growing cycle, a day before harvest, rose geranium plants were mechanically wounded by cutting off all the apical dominance, and roughly shattering the foliage material using a hand-held designed flail (Fugate et al., 2016). The mechanical wounding was applied uniformly across all plants. Plants were then harvested on the last seventh day after CK treatment, following the same procedure as in the first growing cycle.

During the third last growing cycle, plants were subjected to moisture stress, seven days before harvest. All plants were uniformly subjected to moisture stress using polyethylene glycol (PEG 6000) at -0.15 MPa of osmotic stress. To reach the -0.15 MPa of osmotic pressure, PEG 6000 was diluted in the nutrient solution by adding 100g/L (Sedibe and Allemann, 2011). Plants were then harvested on the last seventh day after CK treatment, following the same procedure as in the first and second growing cycles.

Growing conditions. Plants were fertigated three times per day (8:00 am, 12:00 pm, and 4:00 pm) using a 'drain to waste' drip irrigation system, where each dripper supplied 2 L/h of water and nutrient solution (Sedibe and Allemann, 2012). Initially, fertigation intervals for the young plants lasted for five minutes, containing a balanced nutrient solution, as recommended for rose geranium (Table 1a and 1b).

Table 1a. Recommended macronutrient (ions) composition used to fertigate rose geranium plants in this experiment

Ions (meq/L)	Concentrations
NH_4^+	0.91
K^+	5.35
Ca^{2+}	5.85
Mg^{2+}	2.36
NO_3^-	9.18
$H_2PO_4^-$	2.09
SO_4^{2-}	3.20

Table 1b. Recommended micronutrient composition used to fertigate rose geranium plants in this experiment

Micronutrient (mg/L)	Chemical form	Concentrations
Fe	Libfer; 13% Fe-EDTA	1.12
Mn	Manganese sulphate	0.54
Zn	Zinc sulphate	0.18
B	Boric acid	0.03
Cu	Copper sulphate	0.02
Mo	Ammonium molybdate	0.05

As the plants matured and the demand for water and nutrients increased, the applied volumes were increased to ensure that ca. 10% to 25% of the solution drained to waste, to prevent salt accumulation in the growing media (Combrink, 2005; Sedibe and Allemann, 2012). The electric conductivity (EC) and pH of the nutrient solutions were maintained at 1.6 mS/cm and 5.5, respectively. Using a pH and EC meter (Hanna HI 98129 Digital meter), the desired pH and EC levels were achieved by using nitric acid and adjusting the nutrient solution concentration to reach the desired EC (Combrink, 2005). Malasol was preventatively sprayed for three to six days, at four-week intervals, at 1.75 ml/L throughout the cropping seasons.

Parameters. After three full months, the harvesting of the crops took place and five fully grown plants per experimental unit were cut at a height of ±15 cm above ground level (Araya, 2012) and placed tenderly on the paper towel to remove any remains of free surface water. The weighing of the herbage mass was conducted rapidly before wilting could occur. By weighing the fresh plant materials making use of a PGL 2002 Adam Scale as described in the procedures of Wood and Roger (2000), the fresh herbage mass (FHM) was determined.

A custom-made distiller with a capacity of 5kg was utilized to extract the essential oil from the herbage material of rose geranium (Khetsha et al., 2021). The plant material lifted per treatment unit for oil extraction was then distilled for one hour at a temperature of ±98°C. The total oil yield per treatment was computed after weighing the oil quantity with a PGL 2002 Adam scale (USA) as soon as possible after the distillation and separation processes (Swamy and Rao, 2009).

The extracted essential oil was analysed using gas chromatography (GC) (Agilent 7890B), equipped with a 30 mm x 0.25 mm x 0.25 µm column (Agilent 19091S 433 UI, HP5-MS UI) and a mass selective detector (Agilent 5977A). The oven temperature program was maintained at 60°C for 10 mins. The temperature was increased to 100°C at a rate of 2°C/min, and then to 145°C at a rate of 1°C/min. Finally, the temperature was increased to 300°C at a rate of 20°C/min and then run for two mins. Helium was used as carrier gas at a constant flow of 0.67 ml/min. Spectra were obtained by electron impact at 70 eV, scanning from 35/mz to 550/mz. The peak areas of the selected GC constituents were individually expressed as percentages of the total of all the Total Ion Chromatogram (TIC) peak areas, as determined by mass spectrometry detection (MSD at 250°C) without using correction factors, illustrated in Figure 2 (a and b) for the two varieties. The compounds were identified using the NIST11 mass spectral library. The ISO standard (ISO 4731E, 2012) was used to characterise rose geranium (Reunion-type and Madagascar-type cv.) essential oil quality parameters for the perfumery industry (Table 2).

Table 2. Chromatographic profiles of Reunion-type and Madagascar-type commercially recognised essential oil compounds according to ISO 4731[E] (2012) with retention time

Component	Retention time (min)	[1]Min. %	[2]Max. %	Min. %	Max. %
		Reunion-type		Madagascar-type	
cis-Rose oxide	14.85	0.3	1.1	0.4	1.4
trans-Rose oxid	15.85	0.1	0.5	0.1	0.6
Isomenthone	17.73	5.0	10.0	5.0	10.0
Geranyl formate	25.37	4.0	8.0	3.7	7.0
[3]Citro. formate	25.46	6.5	11.0	6.5	11.0
Guaia-6,9-diene	38.90	5.0	8.5	5.0	9.0
Geranyl butyrate	50.78	0.7	2.0	0.7	1.7
Geranyl tiglate	64.84	0.7	2.0	0.7	2.0
Citronellol	22.37	18.0	26.0	18.0	26.0
Geraniol	24.00	12.0	20.0	10.0	20.0
Linalool	14.30	8.0	11.0	4.0	10.0
[4]β-Phen. tiglate	52.77	0.4	1.0	0.4	1.0

[1]Min. = Minimum; Max. = Maximum; [3]Citro. formate = Citronellyl formate; [4]β-Phen. tiglate = β-phenylethyl tiglate.

Data analysis. The essential oil yield parameters, major essential oil compounds and the essential oil quality-determining compounds were statistically analysed and compared using PROC GLIMMIX, SAS version 9.4 (PROC GLIMMIX, SAS Institute). Significantly different means among the treatments were separated using Tukey's least significant difference *ad hoc* mean comparison tests, at the 0.05 level of significance (Steel and Tourie, 1980). The Shapiro-Wilks test was performed on standardised residuals to test for any deviations from normality (Shapiro and Wilk, 1965). Only the total essential oil composition was subjected to multivariate data analysis, using principal component analysis (PCA) (PCA-XLSTAT 2015) to identify and evaluate the groupings between the variables.

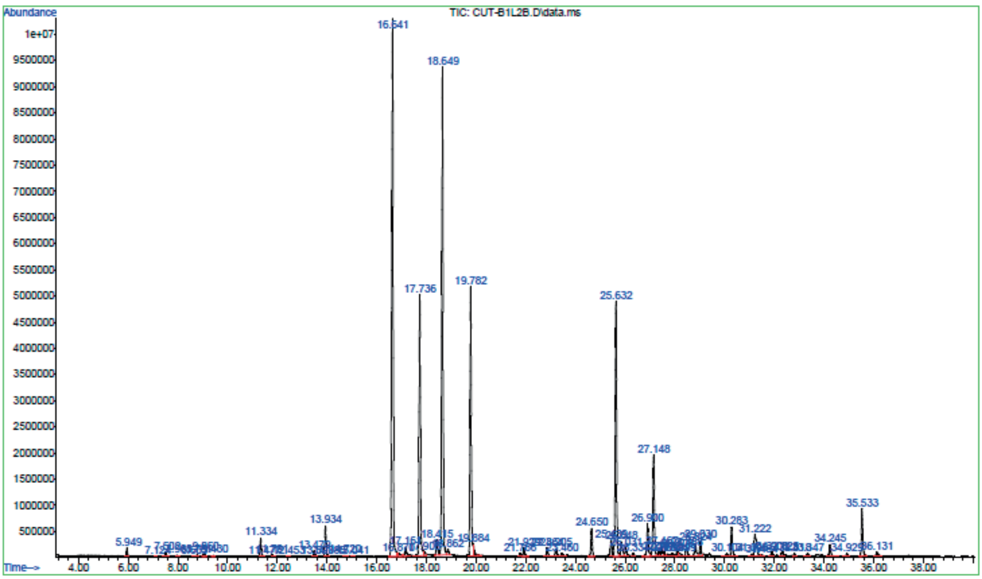

Figure 2a. Total chromatograms of rose geranium essential oil profile for the Reunion-type cv

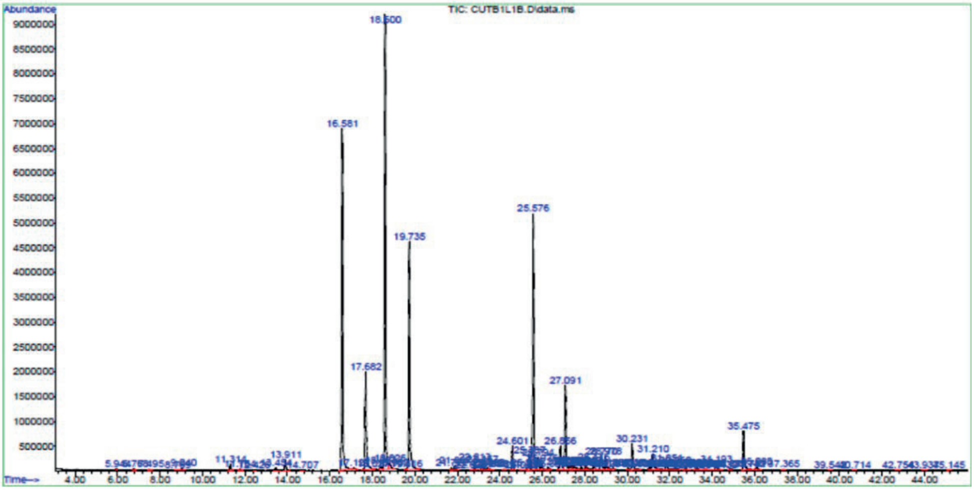

Figure 2b. Total chromatograms of rose geranium essential oil profile for the Madagascar-type cv

RESULTS AND DISCUSSIONS

Essential oil yield parameters. Foliar herbage mass, essential oil mass, and content were all affected by the interactions between rose geranium varieties and the CK application in all production cycles (Table 3).

Table 3. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on the essential oil yield parameters for three production cycles, November 2018 - January 2019; February 2019 - April 2019; May 2019 - July 2019

Treatments	Essential oil yield parameters								
	November 2018 - January 2019			February 2019 - April 2019			May 2019 - July 2019		
	[1]FHM	Oil mass	Oil %	FHM	Oil mass	Oil %	FHM	Oil mass	Oil %
Cytokinin (μM)									
Control	350.91^{b}	0.10^{d}	0.04^{d}	261.44^{d}	0.23^{a}	0.10^{c}	210.31^{d}	0.12^{c}	0.06^{c}
221.6	506.55^{a}	0.37^{c}	0.09^{c}	337.40^{c}	0.68^{d}	0.20^{b}	291.24^{c}	0.56^{c}	0.22^{b}
443.2	503.66^{a}	0.94^{b}	0.20^{b}	497.55^{b}	0.95^{c}	0.24^{b}	328.81^{b}	0.93^{b}	0.30^{a}
664.8	516.03^{a}	1.25^{a}	0.26^{a}	402.76^{a}	1.50^{b}	0.32^{a}	448.45^{a}	1.22^{a}	0.30^{a}
886.4	520.64^{a}	1.25^{a}	0.24^{a}	504.70^{a}	1.31^{a}	0.25^{b}	458.36^{a}	1.28^{a}	0.32^{a}
LSD	37.54	0.15	0.04	34.45	0.10	0.04	35.99	0.20	0.07
P-value	0.005	0.001	0.001	0.003	0.001	0.001	0.002	0.001	0.005
Variety									
Reunion	404.75^{b}	1.02^{a}	0.23^{a}	360.32^{b}	1.10^{a}	0.29^{a}	287.92^{b}	1.13^{a}	0.36^{a}
Madagascar	568.90^{a}	0.58^{b}	0.10^{b}	454.08^{a}	0.73^{b}	0.15^{b}	420.98^{a}	0.55^{b}	0.12^{b}
LSD	23.69	0.09	0.02	21.74	0.06	0.03	22.71	0.13	0.04
P-value	0.001	0.004	0.001	0.001	0.003	0.001	0.001	0.003	0.001
Cytokinin x Variety									
ControlxReunion	272.68^{g}	0.16^{f}	0.06^{e}	224.81^{f}	0.31^{f}	0.14^{f}	186.50^{e}	$0.16^{e,f}$	0.08^{d}
ControlxMadagascar	$468.25^{d,e}$	0.02^{f}	0.00^{f}	316.38^{e}	0.10^{g}	0.04^{g}	$246.02^{c,d}$	0.07^{f}	$0.03^{c,d}$
221.6xReunion	379.51^{f}	0.54^{e}	$0.14^{c,d}$	331.70^{e}	0.81^{d}	$0.24^{c,d}$	$222.85^{d,e}$	0.72^{c}	0.33^{b}
221.6xMadagascar	633.59^{a}	0.19^{f}	$0.03^{e,f}$	$343.10^{d,e}$	0.54^{e}	$0.16^{e,f}$	359.62^{c}	$0.40^{d,e}$	$0.11^{c,d}$
443.2xReunion	$423.97^{e,f}$	1.15^{b}	0.27^{b}	$386.82^{c,d}$	1.03^{c}	0.27^{c}	296.78^{b}	1.25^{b}	$0.42^{a,b}$
443.2xMadagascar	$583.36^{a,b}$	$0.74^{d,e}$	0.13^{d}	$418.69^{b,c}$	0.82^{d}	$0.21^{c,d,e}$	360.83^{b}	$0.61^{c,d}$	0.17^{c}
664.8xReunion	446.09^{e}	1.71^{a}	0.38^{a}	441.21^{b}	1.97^{a}	0.45^{a}	369.38^{b}	1.68^{a}	0.46^{a}
664.8xMadagascar	$585.96^{a,b}$	$0.78^{c,d}$	$0.13^{c,d}$	553.89^{a}	1.02^{c}	$0.18^{d,e,f}$	527.51^{a}	0.75^{c}	0.14^{c}
886.4xReunion	$501.48^{c,d}$	1.51^{a}	0.30^{b}	$417.09^{b,c}$	1.51^{b}	0.34^{b}	364.10^{b}	1.82^{a}	0.50^{a}
886.4xMadagascar	$539.79^{b,c}$	$0.98^{b,c}$	0.18^{c}	592.41^{a}	1.12^{c}	$0.15^{e,f}$	552.61^{a}	0.73^{c}	0.13^{c}
LSD	53.35	0.21	0.05	48.96	0.14	0.06	51.14	0.28	0.10
P-value	0.002	0.005	0.002	0.006	<.0001	0.008	0.005	0.003	0.002

[1]FHM = Foliar Herbage Mass

The foliar application of CK at any level between the two varieties, Reunion-type and Madagascar-type was found to differ significantly in FHM, essential oil mass and content (±P<0.01) in all three growing cycles. For example, during the first production cycle (November 2018 - January 2019), the FHM was recorded significantly higher when CK-221.6 µM was administered on the Madagascar type compared to the control and the Reunion type. However, the significantly higher FHM where CK-221.6 µM was administered did not differ significantly from CK-443.2 and -664.8 µM. The lowest FHM was recorded where CK-221.6 µM was administered on the Reunion-type. In the second growth cycle (February 2019 - April 2019), the Madagascar type was significantly higher when CK was administered from CK-664.8 to -886.4 µM compared to the control.

A similar trend was recorded in the second growing cycle and during the third growing cycle (May 2019 - July 2019). In this study, it could be observed that administering CK significantly increases the FHM; however, the effects of simulated wounding and stress required high concentrations of CK to record higher herbage mass.

According to Verma et al. (2016), based on the two distinct geranium oil types, the African type, and the Reunion type, the two varieties in this study, did not possess 10-epi-γ-eudesmol; thus, they possess the same traits; Reunion type. Tembe and Deodhar (2010a), Motsa et al. (2006), Gupta et al. (2002) and Van Der Walt and Dermane (1988) reported the distinct morphological differences between the Reunion type and the Bourbon type, where the Reunion type possessed long and fine-textured bristles with distinct deep-lobed fan shape containing at least five to seven divided lobes. Therefore, high FHM in all production cycles in this study could first be attributed to the leaf morphology since the Madagascar type is highly associated with the Bourbon type morphologically (Juliani et al., 2006). It has been reported widely that CK increases the herbage material of aromatic plants such as rose geranium. For example, El-Sayed et al. (2023) reported enhanced vegetative growth when CK was applied in the form of benzyladenine on *P. zonale* (L.) L'Hér. ex Aiton. With amino acids, CK increased the development of the uniform growth of shoots in *P. hederaefolium* (spp.) and *P. hotorum* (spp.); subsequently increasing the herbage material (Wojtania and Gabryszewska, 2001). On lemongrass (*Cymbopogon citratus* (DC.) Stapf.), when CK was applied, the plants exhibited highly branching morphology (Camas-Reyes et al., 2022). Shoot regeneration in ciplukan (*Physalis angulata* L.) has been induced by supplementation with different types of CK (Mastuti et al., 2017).

As illustrated in Table 3, the essential oil mass was recorded significantly (±P<0.01) higher when CK was administered on the Reunion type compared to the control and the Madagascar type in all production cycles. Interestingly, the highest recorded was when the CK was applied at a concentration of CK-664.8 µM in all production cycles; however, when the CK applications were increased to CK-886.4 µM in the first and third growing cycles, the essential oil did not change significantly. It could be deduced that an application of CK-664.8 µM improves the essential oil yield, irrespective of any stress-induced closer to harvest. Farooqi and Sharma (1988) and Khetsha et al. (2021) reported that the administration of CK (kinetin) increased the essential oil yield of Japanese mint (*Mentha arvensis* (L.) var. piperascens Mal.), and rose geranium, respectively; however, the applications were much lesser compared to the administered CK in this study. On the other hand, during the second production cycle, a day before harvest, all rose geranium plants were uniformly mechanically wounded by cutting off all the apical dominance, and roughly shattering the foliage material using a hand-held designed flail (Fugate et al., 2016). The increased essential oil yield during the second growing cycle when CK was applied at CK-886.4 µM could be attributed to the interaction between CK and simulated wounding. When plants have endured wounding, the physiological regeneration mechanism of the shoots is dependent on the interaction between exogenous CKs, brassinosteroids, auxin and gibberellic acid, (Nanda and Melnyk, 2018); therefore, in this study, upregulation of CK (886.4 µM) in synergistic relation with other plant growth regulators may have improved the essential oil yield at this concentration.

Essential oil content is another important parameter used to determine the essential oil yield based on the FHM and the essential oil mass (Table 3). In this study, the essential oil content was similar to the findings recorded in the essential oil in the first two production cycles. As shown in Table 3, 0.38 and 0.45% essential oil contents were recorded when CK-664.8 μM was administered. During the last production cycle, the highest essential oil content (0.5%) was recorded when CK-886.4 μM was administered; however, this was not significantly different compared to CK-664.8 μM therefore, it could be deduced that an administration of CK-664.8 μM could be recommended for the essential oil yield (essential oil mass and content) on Reunion-type. As widely reported, the Reunion-type bears smaller shrub compared to a majority of rose geranium varieties and yet produces significantly higher essential oil yield (Tembe and Deodhar, 2010a), which were similar findings in this study. El-Keltawi and Croteau (1987) reported partially similar results, where different types of CK were administered on *M. piperita* (L.), *M. spicata* (L), *M. suaveolens* (Ehrh.) and *Salvia officinalis* (L.), improving biosynthesis of essential oil; in this case, increasing the yield and essential oil content.

Major essential oil compounds. Table 2 and Figure 2 depict the chromatographic profiles of Reunion-type and Madagascar-type commercially recognised essential oil compounds and the C:G ratio (essential oil quality determining parameter for aromatic and perfumery industry) according to ISO 4731[E] (2012) with retention time. Therefore, all essential oil compounds discussed in this study were based on the accepted indexes as described in Table 2, with accepted concentrations ranging from minimum to maximum indexes. Tables 4, 5 and 6 illustrate the major essential oil compounds for production cycles from November 2018 to January 2019; February 2019 to April 2019; and May 2019 to July 2019, respectively.

Table 4. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the November 2018 to January 2019 production cycle

Treatments	Major essential oil compounds November 2018 – January 2019								
	cis-Rose oxide	*trans*-Rose oxide	[1]Isom.	Guaia-6,9-diene	[2]*β*-Phen. tiglate	Citronellyl formate	Geranyl formate	Geranyl butyrate	Geranyl tiglate
Cytokinin (μM)									
Control	0.12[a,b]	0.05[a]	6.26[b]	11.78[a]	1.13[a]	18.89[a]	7.37[a]	0.63[b]	1.19[a]
221.6	0.13[a]	0.06[a]	7.38[a]	12.22[a]	1.29[a]	19.33[a]	7.00[a]	0.68[a,b]	1.44[a]
443.2	0.10[a,b]	0.04[a]	7.20[a]	11.96[a]	1.32[a]	19.31[a]	7.01[a]	0.82[a,b]	1.24[a]
664.8	0.13[a]	0.06[a]	8.03[a]	12.28[a]	1.69[a]	20.47[a]	6.81[a]	0.96[a]	1.41[a]
886.4	0.09[b]	0.04[a]	7.73[a]	12.76[a]	1.23[a]	20.31[a]	7.56[a]	0.83[a,b]	1.31[a]
LSD	0.04	0.02[a]	0.92	1.44	0.43	1.47	0.83	0.22	0.28
P-value	0.07	0.33[a]	0.01	0.65	0.12	0.16	0.30	0.04	0.34
Variety									
Reunion	0.13[a]	0.06[a]	6.66[b]	12.41[a]	1.00[b]	19.22[b]	6.62[b]	0.83[a]	1.19[b]
Madagascar	0.09[b]	0.04[b]	8.11[a]	12.01[a]	1.71[a]	20.19[a]	7.70[a]	0.75[a]	1.45[a]
LSD	0.02	0.01	0.58	0.91	0.27	0.93	0.53	0.14	0.18
P-value	0.01	0.02	0.01	0.31	<.0001	0.05	0.01	0.22	0.01
Cytokinin x Variety									
ControlxReunion	0.15[a]	0.06[a]	5.27[a]	12.07[a]	0.96[a]	18.72[a]	7.06[a]	0.57[a]	1.09[a]
ControlxMadagascar	0.08[a]	0.04[a]	7.75[a]	11.35[a]	1.39[a]	19.15[a]	7.83[a]	0.72[a,b]	1.33[a]
221.6xReunion	0.16[a]	0.07[a]	6.47[a]	11.99[a]	0.63[a]	19.63[a]	6.38[a]	0.59[b]	1.28[a]
221.6xMadagascar	0.10[a]	0.04[a]	8.30[a]	12.45[a]	1.95[a]	19.04[a]	7.63[a]	0.77[a,b]	1.59[a]
443.2xReunion	0.11[a]	0.05[a]	6.50[a]	12.21[a]	1.00[a]	19.46[a]	6.40[a]	0.87[a,b]	1.11[a]
443.2xMadagascar	0.08[a]	0.03[a]	7.90[a]	11.70[a]	1.64[a]	19.17[a]	7.62[a]	0.78[a,b]	1.36[a]
664.8xReunion	0.13[a]	0.06[a]	8.10[a]	13.01[a]	1.49[a]	18.91[a]	6.41[a]	1.18[a]	1.23[a]
664.8xMadagascar	0.13[a]	0.06[a]	7.97[a]	11.54[a]	1.90[a]	22.02[a]	7.21[a]	0.74[a,b]	1.58[a]
886.4xReunion	0.10[a]	0.05[a]	6.97[a]	12.75[a]	0.91[a]	22.02[a]	6.87[a]	0.93[a,b]	1.26[a]
886.4xMadagascar	0.07[a]	0.04[a]	8.50[a]	12.77[a]	1.55[a]	21.23[a]	8.25[a]	0.73[a,b]	1.36[a]
LSD	0.06	0.03	1.31	2.05	0.61	2.08	1.19	0.31	0.40
P-value	0.34	0.60	0.11	0.66	0.22	0.08	0.95	0.04	0.89

[1]Isom. = Isomenthone.

[2]*β*-Phen. tiglate = *β*-phenylethyl tiglate

Table 5. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the February 2019 - April 2019 production cycle

Treatments	Major essential oil compounds February 2019 – April 2019							
	cis-Rose oxide	*trans*-Rose oxide	Isomenthone	Guaia-6,9-diene	[1]*β*-Phen. tiglate	Citronellyl formate	Geranyl formate	Geranyl tiglate
Cytokinin (μM)								
Control	0.09^{a}	0.08^{a}	11.84^{a}	11.87^{a}	0.78^{a}	21.17^{a}	6.40^{b}	1.31^{c}
221.6	0.05^{a}	0.02^{a}	10.95^{a}	13.01^{a}	1.16^{a}	$18.57^{a,b}$	10.78^{a}	2.85^{a}
443.2	0.09^{a}	0.04^{a}	8.03^{a}	13.52^{a}	1.08^{a}	$18.87^{a,b}$	10.07^{a}	2.86^{a}
664.8	0.05^{a}	0.02^{a}	9.21^{a}	13.53^{a}	1.11^{a}	18.20^{b}	10.82^{a}	2.15^{b}
886.4	0.05^{a}	0.02^{a}	5.76^{a}	14.05^{a}	1.26^{a}	16.40^{b}	11.07^{a}	3.18^{a}
LSD	0.05	0.06	6.48	1.47	0.44	2.76	1.25	0.55
P-value	0.28	0.22	0.35	0.08	0.50	0.04	<.0001	0.001
Variety								
Reunion	0.08^{a}	0.04^{a}	9.14^{a}	12.58^{b}	0.92^{b}	19.81^{a}	9.52^{a}	2.03^{b}
Madagascar	0.05^{a}	0.03^{a}	8.99^{a}	13.95^{a}	1.26^{a}	17.21^{b}	10.37^{a}	3.02^{a}
LSD	0.03	0.04	4.09	0.92	0.28	1.74	0.79	0.35
P-value	0.06	0.44	1.00	0.01	0.01	0.009	0.09	<.0001
Cytokinin x Variety								
ControlxReunion	0.07^{a}	0.09^{a}	$7.85^{b,c,d}$	12.03^{a}	0.80^{a}	21.10^{a}	6.37^{a}	0.73^{e}
ControlxMadagas car	0.11^{a}	0.06^{a}	17.83^{a}	11.62^{a}	0.75^{a}	21.27^{a}	6.44^{a}	2.17^{e}
221.6xReunion	0.06^{a}	0.02^{a}	$6.23^{c,d}$	12.62^{a}	0.87^{a}	19.68^{a}	10.59^{a}	$2.91^{b,c}$
221.6xMadagascar	0.04^{a}	0.03^{a}	$15.67^{a,b}$	13.40^{a}	1.44^{a}	17.45^{a}	11.07^{a}	$2.79^{c,d}$
443.2xReunion	0.14^{a}	0.04^{a}	$9.87^{a,b,c,d}$	12.17^{a}	0.76^{a}	20.88^{a}	9.67^{a}	$2.27^{b,c}$
443.2xMadagascar	0.03^{a}	0.03^{a}	$6.20^{c,d}$	14.86^{a}	1.39^{a}	16.86^{a}	10.48^{a}	$3.44^{c,d}$
664.8xReunion	0.05^{a}	0.02^{a}	$12.53^{a,b,c}$	12.80^{a}	1.02^{a}	19.43^{a}	10.39^{a}	$1.44^{a,b}$
664.8xMadagascar	0.04^{a}	0.03^{a}	$5.88^{c,d}$	14.25^{a}	1.19^{a}	16.98^{a}	11.25^{a}	2.86^{c}
886.4xReunion	0.04^{a}	0.02^{a}	$9.22^{a,b,c,d}$	13.26^{a}	1.14^{a}	17.95^{a}	10.60^{a}	2.82^{a}
886.4xMadagascar	0.06^{a}	0.03^{a}	2.30^{d}	14.84^{a}	1.37^{a}	14.84^{a}	11.53^{a}	3.54^{a}
LSD	0.08	0.08	9.21	2.08	0.63	3.92	1.79	0.78
P-value	0.11	0.91	0.02	0.35	0.67	0.64	0.93	0.03

[1]*β*-Phen. tiglate = *β*-phenylethyl tiglate

Table 6. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the May 2019 - July 2019 production cycle

Treatments	Essential oil compounds May 2019 – July 2019						
	cis-Rose oxide	*trans*-Rose oxide	Isomenthone	Guaia-6,9-diene	Citronellyl formate	Geranyl formate	Geranyl tiglate
Cytokinin (μM)							
Control	0.08^{a}	0.05^{a}	14.20^{a}	13.72^{a}	17.41^{a}	5.92^{a}	2.78^{a}
221.6	0.10^{a}	0.05^{a}	12.47^{a}	13.55^{a}	17.34^{a}	5.86^{a}	2.69^{a}
443.2	0.11^{a}	0.06^{a}	10.90^{a}	14.04^{a}	18.04^{a}	5.77^{a}	2.61^{a}
664.8	0.10^{a}	0.06^{a}	13.77^{a}	14.31^{a}	16.84^{a}	5.99^{a}	2.79^{a}
886.4	0.09^{a}	0.06^{a}	9.08^{a}	14.52^{a}	16.56^{a}	5.67^{a}	2.72^{a}
LSD	0.05	0.03	9.43	1.38	2.55	1.49	0.58
P-value	0.83	0.94	0.76	0.49	0.73	0.98	0.97
Variety							
Reunion	0.11^{a}	0.06^{a}	12.96^{a}	13.47^{b}	18.05^{a}	5.53^{a}	2.44^{b}
Madagascar	0.08^{a}	0.05^{a}	10.99^{a}	14.65^{a}	16.36^{a}	6.17^{a}	3.01^{a}
LSD	0.03	0.02	5.95	0.87	1.61	0.94	0.36
P-value	0.13	0.24	0.54	0.02	0.06	0.13	0.005
Cytokinin x Variety							
ControlxReunion	0.09^{a}	0.05^{a}	12.17^{a}	13.06^{a}	18.47^{a}	5.91^{a}	2.42^{a}
ControlxMadagas car	0.08^{a}	0.05^{a}	17.25^{a}	14.71^{a}	15.84^{a}	5.59^{a}	3.32^{a}
221.6xReunion	0.11^{a}	0.05^{a}	9.23^{a}	12.89^{a}	18.63^{a}	5.47^{a}	2.49^{a}
221.6xMadagascar	0.08^{a}	0.04^{a}	15.70^{a}	14.20^{a}	16.05^{a}	6.25^{a}	2.88^{a}
443.2xReunion	0.15^{a}	0.07^{a}	12.70^{a}	12.83^{a}	20.29^{a}	5.95^{a}	2.11^{a}
443.2xMadagascar	0.07^{a}	0.04^{a}	9.10^{a}	15.26^{a}	15.79^{a}	5.59^{a}	3.11^{a}
664.8xReunion	0.10^{a}	0.05^{a}	18.43^{a}	14.14^{a}	17.15^{a}	6.48^{a}	2.50^{a}
664.8xMadagascar	0.10^{a}	0.06^{a}	9.10^{a}	14.48^{a}	16.53^{a}	6.48^{a}	3.08^{a}
886.4xReunion	0.09^{a}	0.06^{a}	12.27^{a}	14.43^{a}	15.72^{a}	4.84^{a}	2.67^{a}
886.4xMadagascar	0.09^{a}	0.05^{a}	5.90^{a}	14.61^{a}	17.40^{a}	6.49^{a}	2.77^{a}
LSD	0.07	0.04	13.40	1.97	3.63	2.11	0.82
P-value	0.36	0.24	0.33	0.44	0.16	0.68	0.52

Rose oxide (*cis*- and *trans*-), guaia-6,9-diene, *β*-phenylethyl tiglate, citronellyl formate, and geranyl formate were not affected by interactions between rose geranium varieties and the CK in all production cycles (Tables 4, 5 and 6).

During the first growing cycle, both Rose oxide compounds, *cis*- and *trans*- were significantly affected by the variety. In both compounds, *cis*-Rose oxide, and *trans*-Rose oxide, Reunion-type recorded 0.04% (P<0.01) and 0.02% (P<0.02) higher compared to the Madagascar-type.

According to ISO 4731[E] (2012) (Table 2), both compounds were above the index range, recording 0.13% and 0.06; thus, the compounds may not be considered for use in the high-quality perfumery industry. As illustrated in Tables 5 and 6, the two compounds were not significantly different for the two growing cycles; however, a similar trend was recorded, and at most, these compounds were within the range required for the industry during these periods.

Isomenthone compound was recorded significantly (P<0.01) lower where plants were treated with distilled water compared to all CK treatments; therefore, confirming that any application of CK improves the isomenthone content in both varieties (Table 4). In a study conducted by Zielińska et al. (2011) on *Agastache rugosa* (Fischer & C.A.Meyer) O. Kuntze, administering CK in the form of kinetin showed less differences between the treatments on isomenthone content; however, these treatments were better than the control. On the other hand, the Madagascar type recorded a significantly higher content of isomenthone compared to the Reunion type, and both varieties were within the range as described in ISO 4731[E] (2012) (Table 4). Significant interactions between rose geranium varieties and the CK application were recorded during the second growing cycle; however, data between the interactions were inconsistent to conclude (Table 5). No significant effects, including the interactions were recorded for the third growing cycle for isomenthone (Table 6).

As illustrated in Tables 5 and 6, only the varieties were recorded as significant for the guaia-6,9-diene compound; however, this was not the case during the first growing cycle, Table 4. Madagascar-type had the highest (P<0.01) guaia-6,9-diene content compared to Reunion-type during the second growing cycle, with the same recording (P<0.02) during the third growing cycle, respectively. During these production cycles, the content of guaia-6,9-diene recorded was higher than the recommended ISO 4731[E] (2012); therefore, this compound could mostly be considered for other aromatic and cleaning industries, not the perfumery industry.

During the two cycles where *β*-phenylethyl tiglate was recorded, the variety was the only factor significantly affected. In both growing cycles one (P<.0001) and two (P<0.01), Madagascar-type recorded higher *β*-phenylethyl tiglate content; however, the content was higher to be recommended for the perfumery industry (ISO 4731[E], 2012). Amongst the treatments applied, *β*-phenylethyl tiglate was recorded in traces during the last treatments, and therefore this compound was excluded in Table 6.

In the second and third production cycles, simulated wounding and moisture stress were introduced closer to harvest. Da Silva et al. (2023) and Khetsha and Sedibe (2015) reported that various abiotic and biotic factors like moisture stress, salinity, temperature, heavy minerals, wounding, defoliation, light, and living organisms significantly affect the biosynthesis of essential oil compounds. Therefore, the increased content of guaia-6,9-diene and *β*-phenylethyl tiglate in Madagascar-type could be attributed to the two stress factors induced closer to harvest, the simulated wounding, and moisture stress during the second and third growing cycles, respectively. There are no published articles to corroborate the differences in guaia-6,9-diene and *β*-phenylethyl tiglate in varieties of rose geranium.

The administration of CK at any concentration significantly reduced the citronellyl formate content (Table 5). Citronellyl formate content was recorded as significantly (P<0.01) higher in the Madagascar type compared to the Reunion type during the first growing cycle. However, this was not the case during the second growing cycle: citronellyl formate content tended to be 0.97% significantly (P<0.009) higher in the Reunion type compared to the Madagascar type (Table 5). Nonetheless, the citronellyl formate content was higher than the recommended content, ISO 4731[E] (2012). There were no significant differences recorded in the citronellyl formate content during the third growing cycle (Table 6). The Madagascar type and the Reunion type resemble the same requirements of between 6.5 and 11% of citronellyl formate content (ISO 4731[E], 2012); thus, changes between the two varieties could be attributed to the uniform simulated wounding close to the harvest. The results of this study demonstrated that the Madagascar type may be sensitive to any type of wounding closer to harvest, especially to produce citronellyl formate. On the other hand, the Reunion type produced a higher content of citronellyl formate when subjected to simulated wounding closer to harvest, indicating a favourable strategy to produce citronellyl formate; nonetheless, growers need to be cautious as the strategy tends to increase the content of citronellyl formate beyond the acceptable index for perfumery industry. Steele et al. (1998) reported that monoterpene synthases arise earlier when grand fir (*Abies grandis* (Douglas ex D. Don) Lindley) endured simulated wounding. Therefore, since citronellyl formate is the monoterpene type present mostly in *P. graveolens* (Džamić et al., 2014), the attributes of the changes in the content of this monoterpene during the second production cycle are attributed to simulated wounding.

According to Khetsha et al. (2022), Liu et al. (2016), and Demyttenaere et al. (2000), geranyl acetate, geranyl butyrate, geranyl formate and geranyl tiglate formed from biotransformation of the geraniol as esters, subsequently changing the essential oil composition and quality. In this study, the geraniol esters were mostly recorded; however, at most, they appeared in traces. For example, during the second and third growing cycles (Tables 5 and 6), geranyl butyrate was recorded in traces amongst the treatments, while geranyl acetate was in traces across all growing cycles; thus, these compounds were excluded.

As illustrated in Table 4, geranyl formate and geranyl tiglate were marginally ($\pm P<0.01$) within the acceptable index as recommended by ISO 4731[E] (2012) in Madagascar-type compared to Reunion-type. Interestingly, as illustrated in Tables 5 and 6, a similar trend was recorded, where the Madagascar-type accumulated significantly ($P<.0001$) higher geranyl tiglate content compared to the Reunion-type. The results of this study showed that irrespective of treatments, Madagascar-type accumulated more geranyl tiglate; however, it could not be associated with any published literature.

Interestingly, when CK was applied to the two varieties, the geranyl tiglate content tended to be significantly ($\pm P<0.03$) higher, because of the interactions recorded (Table 5). The geranyl tiglate content increase only occurred when CK concentration was applied at 886.4 µM compared to the controls. Punpee (2012) reported biosynthesis of CK, where the author described the process occurring through biochemical modification of dimethylallyl diphosphate, which eventually through other metabolic processes yields zeatin ribosides, an important type of CK. In addition, Nogués et al. (2006) reported that geranyl esters and dimethylallyl diphosphate share the same novel chloroplastic pathway, the intermediate methylerythritol phosphate. On the other hand, Lulai et al. (2016) reported a quantifiable level of CK when potatoes (*Solanum tuberosum* L.) were subjected to simulated wounding; justifying the possible effects of the wounding on the metabolomes. Egamberdieva et al. (2017) also reported that the exogenous application of CKs can optimize the endogenous levels of CKs for growth and development and have significant effects on the biosynthesis of essential oils after wounding damage. Therefore, in this study, the significant changes in the geranyl tiglate content are associated with high CK concentration application and the uniform simulated wounding closer to the harvest for the two varieties.

The recorded significant ($\pm P<0.01$) interactions between rose geranium varieties and the CK application of geranyl butyrate were not consistent for a meaningful conclusion (Table 4). During the second cycle, irrespective of the CK application concentration, the geranyl formate tended to be significantly better compared to the control (Table 5); this was not the case for the third cycle, no significant differences were recorded. Although a significant change was recorded during the second growing cycle, it was unclear how the CK affected the geranyl butyrate; nonetheless, attributes could be associated with the simulated wounding, the biosynthesis pathway between the CK and the geranyl esters.

Essential oil quality-determining compounds. The most important compounds commonly used in the perfume industry are linalool, geraniol, and citronellol, with a C:G ratio below 3:1 being preferred by the industry for many of the varieties like Reunion-type and Madagascar-type (ISO 4731[E], 2012; Saxena et al., 2008). As illustrated in Table 7 in this study, significant interactions were recorded between rose geranium varieties and the CK application for linalool, citronellol, geraniol and the C:G ratio; however, this differed between the growing seasons.
In all growing cycles, linalool was recorded as significantly high where CK was administered at 886.4 μM on the Reunion-type compared to the control. However, during the second and third growing, there were no significant differences where CK was administered at 886.4 μM on the Madagascar-type; yet the content of linalool was still better than the control. Interestingly, throughout the three cycles, only during the second production cycle linalool was within the acceptable range for the perfumery industry (ISO 4731[E], 2012). There is an association between wounding and the biosynthesis of linalool (Zhang et al., 2022), as well as enhancement of linalool when CK is administered (Tavares et al., 2004). On lemon (*Citrus jambhiri* Lush.), Yamasaki et al. (2007) reported that wounded rough lemons recorded a linalool content that was 14.5 times higher than that of unwounded rough lemon leaves. Ono et al. (2011) and Tavares et al. (2004) reported that linalool was increased by the administration of CK on basil (*Ocimum basilicum* L.) and lemon balm (*Lippia alba* Mill. N. E. Br.) compared to the control, respectively.
According to Weng et al. (2015), the physical and chemical similarities of citronellol and geraniol compounds make them inseparable. In this study, citronellol content was much higher (P<.0001) in the Reunion type compared to the Madagascar type; however, I noticed that only in the second season citronellol content was within the acceptable range for the perfumery industry (Table 7).
According to Tembe and Deodhar (2010b), the chemical composition of the Reunion type remains the best, with a desirable rosy aroma and considerably better citronellol, geraniol and C:G ratio compared to other varieties, including the Madagascar type. On the other hand, the citronellol within the range could be attributed to the simulated wounding; this was also reported by Yamasaki et al. (2007) on wounded rough lemons. During the last season, the application of CK reduced the content of citronellol significantly (P<.0001) in all treatments for the Reunion-type compared to the control. Findings in this study were similar to results reported by Farooqi et al. (1993), wherein damask rose (*Rosa damascene* Mill.) CK application resulted in an increased essential oil content and citronellol content in the essential oil. This was not the case for the Madagascar type, results were inconsistent across all treatments.

Table 7. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on the essential oil quality-determining compounds parameters for three production cycles, November 2018 - January 2019; February 2019 - April 2019; May 2019 - July 2019

Treatments	Rhodinol and [1]C:G ratio											
	November 2018 - January 2019				February 2019 - April 2019				May 2019 - July 2019			
	[2]Lin.	[3]Citro.	[4]Ger.	C:G	Lin.	Citro.	Ger.	C:G	Lin.	Citro.	Ger.	C:G
Cytokinin (μM)												
Control	5.70^{d}	26.74^{a}	6.93^{c}	3.90^{a}	2.78^{d}	23.54^{a}	8.06^{c}	2.92^{a}	3.14^{d}	23.62^{a}	7.95^{c}	2.98^{a}
221.6	7.39^{c}	27.24^{a}	8.47^{b}	3.25^{b}	6.06^{c}	24.08^{a}	9.16^{b}	2.65^{a,b}	4.65^{c}	21.50^{b}	12.09^{a}	1.78^{b}
443.2	8.57^{b}	26.86^{a}	11.45^{a}	2.37^{c}	6.73^{c}	22.02^{a}	8.94^{b}	2.32^{b,c}	5.22^{c}	21.43^{b}	10.20^{b}	2.12^{b}
664.8	8.48^{b}	27.16^{a}	10.93^{a}	3.02^{b}	8.45^{b}	22.74^{a}	10.68^{a}	2.21^{c}	7.82^{b}	19.89^{c}	10.73^{b}	1.91^{b}
886.4	13.47^{a}	27.03^{a}	8.95^{b}	3.17^{b}	10.83^{a}	22.77^{a}	10.16^{a}	2.28^{c}	12.90^{a}	22.75^{a}	11.24^{a,b}	2.08^{b}
LSD	0.84	2.66	1.54	0.63	0.93	2.81	0.77	0.33	1.26	0.91	1.31	0.34
P-value	0.001	0.99	0.001	0.01	<.0001	0.25	0.001	0.05	<.0001	0.001	0.003	0.05
Variety												
Reunion	9.67^{a}	29.69^{a}	11.22^{a}	2.85^{b}	8.01^{a}	24.60^{a}	10.54^{a}	2.37^{a}	7.28^{a}	22.87^{a}	11.31^{a}	2.11^{a}
Madagascar	7.91^{b}	24.15^{b}	7.52^{b}	3.40^{a}	6.25^{b}	21.32^{b}	8.38^{b}	2.55^{a}	6.44^{b}	20.60^{b}	9.69^{b}	2.19^{a}
LSD	0.53	1.68	0.97	0.40	0.59	1.23	0.49	0·21	0.79	0.58	0.83	0.22
P-value	<.0001	<.0001	0.002	0.01	0.004	<.0001	<.0001	0.06	0.01	<.0001	<.0001	0.22
Cytokinin x Variety												

ControlxReunion	4.75^{e}	29.70^{a}	7.65^{d,e}	3.94^{a,b}	2.83^{e}	24.71^{a}	8.30^{d,e}	2.98^{a}	3.61^{d,e}	26.83^{a}	8.49^{e,f}	3.21^{a}
ControlxMadagascar	7.14^{d}	22.31^{a}	5.84^{e}	3.85^{a,b}	2.70^{e}	21.78^{a}	7.69^{e}	2.83^{a}	2.45^{e}	18.86^{d}	7.14^{f}	2.64^{b}
221.6xReunion	7.59^{d}	31.08^{a}	9.19^{c,d}	3.39^{a,b,c}	8.15^{b}	25.59^{a}	9.77^{c}	2.65^{a}	5.66^{c}	23.62^{b}	12.77^{a}	1.85^{c,d,e}
221.6xMadagascar	7.19^{d}	23.40^{a}	7.76^{d,e}	3.10^{b,c,d}	3.97^{e}	22.58^{a}	8.55^{d,e}	2.64^{a}	3.65^{d,e}	19.38^{d}	11.40^{a,b,c}	1.72^{d,e}
443.2xReunion	11.76^{b}	30.76^{a}	12.95^{b}	2.41^{d,e}	7.98^{b,c}	24.82^{a}	9.96^{c}	2.30^{a}	4.83^{c,d}	21.36^{c}	10.55^{b,c,d}	2.04^{c,d}
443.2xMadagascar	5.37^{e}	22.95^{a}	9.95^{c}	2.32^{d,e}	5.91^{d}	19.22^{a}	8.26^{d,e}	2.35^{a}	5.60^{c}	21.49^{c}	9.86^{c,d,e}	2.19^{b,c,d}
664.8xReunion	10.04^{c}	28.75^{a}	15.73^{a}	1.83^{e}	10.12^{a}	24.08^{a}	13.07^{a}	1.84^{a}	9.28^{b}	18.74^{d}	12.11^{a,b}	1.55^{e}
664.8xMadagascar	6.91^{d}	25.58^{a}	6.13^{e}	4.22^{a}	6.79^{c,d}	21.41^{a}	8.28^{d,e}	2.58^{a}	6.37^{b}	21.04^{c}	9.35^{d,e}	2.27^{c,d,e}
886.4xReunion	14.23^{a}	28.18^{a}	10.55^{c}	2.68^{c,d,e}	10.98^{a}	23.79^{a}	11.42^{b}	2.10^{a}	13.00^{a}	23.80^{b}	12.63^{a}	1.89^{b,c}
886.4xMadagascar	12.70^{b}	25.89^{a}	7.34^{d,e}	3.66^{a,b}	10.68^{a}	21.76^{a}	8.90^{c,d}	2.45^{a}	12.79^{a}	21.69^{c}	9.85^{c,d,e}	2.27
LSD	1.20	3.78	2.18	0.90	1.33	4.75	1.10	0.47	1.79	1.30	1.87	0.49
P-value	<.0001	0.09	0.02	0.01	0.001	0.38	0.01	0.07	0.04	<.0001	0.34	0.01

[1]C:G = Citronelol to Geraniol ratio.
[2]Lin. = Linalool.
[3]Citro. = Cintronellol.
[4]Ger. = Geraniol.

The recorded significant (±P<0.01) interactions between rose geranium varieties and the CK application on geraniol were not consistent for a meaningful conclusion during the last growing cycles as illustrated in (Table 7). Therefore, it could be deduced that by applying CK at 664.8 µM following normal production systems and where simulated wounding is administered, the geraniol content may increase. However, when moisture stress is introduced closer to harvest, this is not the case. However, Eiasu et al. (2009) reported that the decline in geraniol is due to the conversion of geraniol into citronellol, and this was also described by Weng et al. (2015).
High-quality essential oil is based on the C:G ratio (ISO 4731[E], 2012; Saxena et al., 2008). In all production cycles, the C:G ratio was best when CK was administered at 664.8 µM on the Bourbon type; however, this interaction was not significantly different during the second growing cycle. The essential oil quality based on the C:G ratio could be associated with the citronellol, geraniol, and CK biosynthesis pathway and the conversions with time as described by Farooqi et al. (1993), Eiasu et al. (2009) and Weng et al. (2015), respectively.
Therefore, in this study, it could be concluded that administration of synthetic CK-664.8 µM closer to harvest following moisture stress and under normal conditions may improve the Rhodinol and essential oil quality. However, it may be beneficial to increase the application level of CK to 886.4 µM following simulated wounding where linalool is targeted on the Bourbon type.

Total essential oil composition. From over 50 organic compounds listed in rose geranium essential oil (Shellie and Marriott, 2003), chromatographic analysis made it possible to identify 39 (74.47% total oil), 44 (91.18% total oil) and 46 (90.94% total oil) compounds for growing cycle one, two, and three, respectively.
The pattern of diversity amongst all essential oil compounds as shown in Figures 3, 4 and 5 was determined using the PCA for each respective production cycle. The grouping of essential oil compounds was affected by the CK and the variety of rose geranium varieties in all growing cycles. Out of nine principal components used in each PCA, the first two principal components (PC1 and PC2) accounted for most of the variability.
The score plot and loading matrix accounted for 61.34%, 63.28%, and 54.93% of the total variances as illustrated in Figures 3, 4 and 5, respectively. In all growing cycles, the two rose geranium varieties were mostly grouped separately (categorization illustrated in black circles in all figures: Figures 3, 4 and 5) irrespective of the CK treatment.
When the CK treatment was considered as a factor, increasing the CK concentration from 221.6 up to 443.2 µM, grouped the Reunion type in the same quadrant; however, this gradual increase in CK application was not different compared to the control (Figure 3). On the other hand, the two higher CK concentrations, 664.8 and 886.4 µM in the Reunion type were grouped separately, indicating significant differences compared to the control and where lower CK was administered. Therefore, increasing CK from 664.8 to 886.4 µM in the Reunion type may improve these essential oil compounds.
In this study, only essential oil compounds contributing most of the variability amongst (squared cosine [r^2]) the essential oils loadings were discussed. Therefore, CK applied at 664.8 and 886.4 µM tended to adversely affect the content of citronellol, *α*-Copaene, (*E*)-*β*-

Caryophyllene, germacrene D, and (*Z*)-Geranyl acetate as illustrated in PC 1 accounting for 43.21% (Figure 3). Findings on the citronellol when PCA was applied assisted with the conclusion on possible adverse effects of CK on rose geranium (Bourbon type) grown under normal conditions.

As illustrated in PC 1 (Figure 3), the Madagascar type was grouped in the same quadrant irrespective of the CK applications (excluding the control). This grouping was shown to increase the content of isomenthone, *α*-Cubebene, *β*-cubenene, *cis*-Muurola-3,5-diene, caryophyllene oxide, and *trans*-Calamenene geranyl tiglate (PC1).

Principal component 2 accounted for 18.12% and grouped *δ*-Cadinene and citronellyl butyrate, which were shown to be increased by any CK treatment except the control for Madagascar type only; thus, administering CK closer to harvest may improve these essential oil compounds of the Madagascar type under normal production system. On the other hand, it was interesting to note that the two varieties bear significantly different essential oil compositions as described by Tembe and Deodhar (2010a) and Tembe and Deodhar (2010b).

During the second cycle, as illustrated in Figure 4, *β*-Myrcene, 3,7-Dimethyl-1,3,6-octatriene, neral, geranial and citronellyl formate content in the Reunion type treated with all CK treatments including the control were all adversely affected as shown in PC 1 (48.36%). Humelene, *α*-Copaene, *β*-Caryophyllene, *trans*-muurola-4(14),5-diene, *α*-muurolene, citronellyl butyrate, 1-epi-cubenol, geranyl tiglate, geranyl butanoate, and citronellyl tiglate content were increased by the increasing the concentration of CK to 886.4 μM on both varieties, Reunion, and Madagascar type. Results on the geranyl tiglate corroborate findings in the analysis of variance, Table 5. On the other hand, increasing CK concentration from 221.6 up to 664.8 μM tended to increase the content of geranyl formate, *α*-Guaiene, guaia-6,9-diene, geranyl propionate, cubenol, germacrene D, 2-Phenylethyl tiglate, geranyl 2-methyl butanoate, and 1,10-di-epi-cubenol on Madagascar type (PC 1). Although the two key compounds were not significantly different when the analysis of variance was applied, the PCA on the other hand showed that geranyl formate and guaia-6,9-diene can be increased by CK concentration from 221.6 up to 664.8 μM.

In PC 2, accounting for a 14.92% level of variation, CK at 886.4 μM increased the content of citronellyl acetate and *β*-Cadinene on both varieties: Reunion and Madagascar type; thus, increasing CK to 886.4 μM improves citronellyl acetate and *β*-Cadinene for the two varieties.

In the third production cycle, PC 1 and PC 2 accounted for 35.61% and 19.32%, respectively (Figure 5). Citronellyl formate and *cis*-Rose oxide were associated with the application of CK at 443.2 μM on the Reunion type; however, this was not significantly different from the control. On the other hand, applying CK at 221.6 and 664.8 μM on the Reunion type adversely affected citronellyl acetate, *β*-Guaiene and *δ*-Cadinene content. There were no significant differences between the CK applied at 221.6, 664.8, and 886.4 μM and the control on Madagascar type.

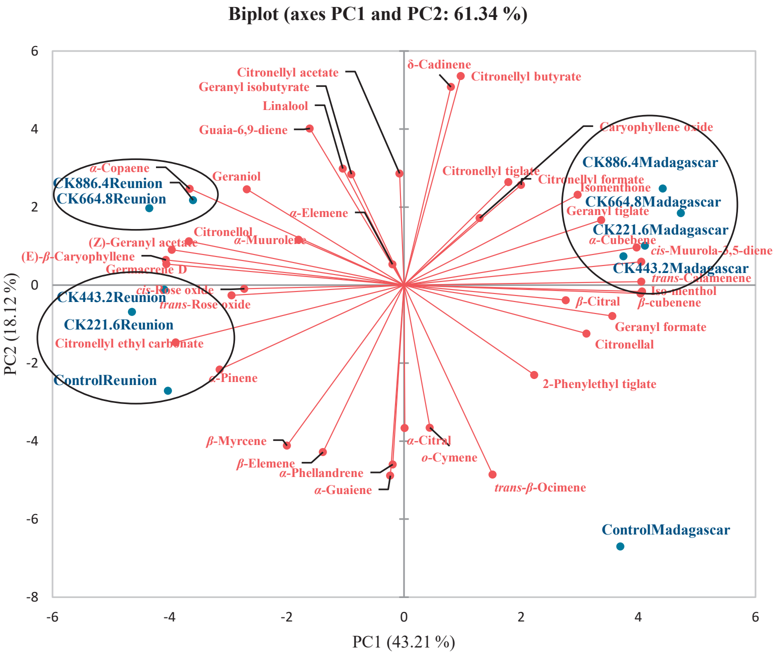

Figure 3. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during the November 2018 to January 2019 production cycle

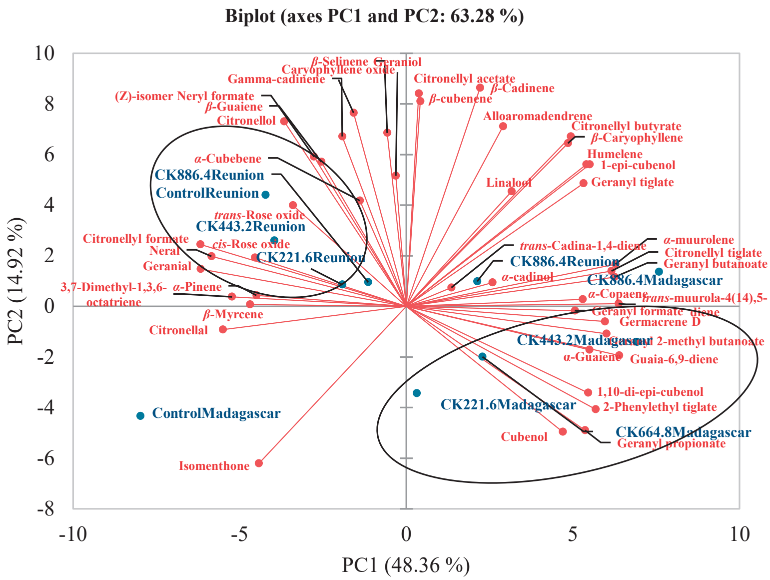

Figure 4. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during February 2019 to April 2019 production cycle

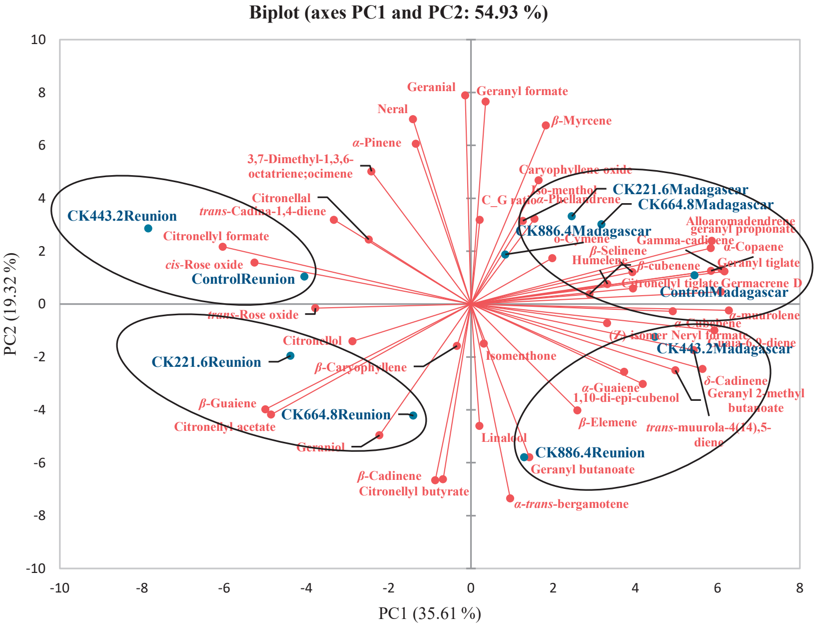

Figure 5. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during the May 2019 to July 2019 production cycle

Interestingly, the highest application of CK (886.4 µM) on Reunion type showed to increase α-Cubebene, guaia-6,9-diene, *trans*-muurola-4(14),5-diene, α-muurolene and geranyl 2-methyl butanoate content. On the other hand, these compounds were also increased by administering CK at 443.2 µM on the Madagascar type.

In PC 2, by applying CK at 221.6, 664.8, 886.4 µM on Madagascar type increased β-Myrcene, geranyl formate, alloaromadendrene, germacrene D, gamma-cadinene and geranyl tiglate; however, this was not different from the control. Administering CK at 443.2 and 886.4 µM on Madagascar and Reunion type, respectively, caused a decline of α-trans-bergamotene, guaia-6,9-diene, *trans*-muurola-4(14),5-diene, α-muurolene, δ-Cadinene and geranyl 2-methyl butanoate content. Based on the analysis of variance, the guaia-6,9-diene was not affected by the interaction between the rose geranium varieties and CK application during the third production cycle; however, when PCA was applied, the two levels were shown to be adverse in the production of guaia-6,9-diene. For this reason, CK may be applied at 221.6 µM for a possible increased content of guaia-6,9-diene in both varieties.

In all production cycles, a trend was observed where uniform simulated wounding with CK administered between 221.6 to 664.8 µM came out to improve most of the essential oil compounds. Although this study focused on the major essential oil compounds and quality, it was interesting to note significant differences between the two varieties and as well how CK improved some of the key compounds, mostly corroborating the analysis of variance.

CONCLUSIONS

Environmental stress factors, such as wounding, temperature, water deficit, salinity and moisture stress have been reported widely to improve the secondary metabolites across wide species. However, this at most caused a significant reduction of the herbage material for aromatic plants such as rose geranium, directly affecting the essential oil yield and quality (Rhodinol).

In this study, the author observed that a uniform simulation of wounding and moisture stress closer to the harvest with CK applied between 221.6 to 664.8 µM concentrations may improve the essential oil yield, quality, and most of the major essential oil compounds for the two varieties: Reunion and Madagascar type cv. In some cases, it may be beneficial to increase the application of CK to 886.4 µM following simulated wounding where linalool and geranyl tiglate content are targeted on the Bourbon type, and Madagascar type, respectively.

Bourbon type remains better than the Madagascar type with the essential oil yield and maintains higher essential oil quality (Tembe and Deodhar, 2010a; Tembe and Deodhar, 2010b); however, both varieties were at most within the high-quality range as prescribed by ISO 4731[E] (2012). Therefore, based on these findings, the author concludes that the administration of any biostimulant containing CK at a concentration of 664.8 µM can be considered by growers, especially closer to harvest to improve the essential oil biosynthesis and quality of both varieties.

Future studies should focus on other cheaper biostimulants containing CK as well as determining the distinctions between normal production systems (as control) and introducing simulated wounding and moisture stress closer to harvest across the three production cycles as a repeated study, as this was the limitation of this study.

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A GREEN APPROACH USED FOR HEAVY METALS REMOVAL FROM HUMAN BODY

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Abstract

Recent research has shown that the level of heavy metals in the human body is near 700 times higher than that of our ancestors. It is known that heavy metals do not degrade and are not broken-down by microorganisms and the period for their elimination from the body is very long. They can accumulate in the liver, brain, kidneys, muscles, bones, nails and hair. The World Health Organization highlights the risks of neurological, renal, liver, heart and bone diseases caused by heavy metals. The treatment for most heavy metal intoxications is chelating therapy, which can extend over a very long period of time and is quite costly. However, complementary treatments with medicinal plants have proven helpful to remove heavy metals by intensify diuresis, purifying the blood, through their depurative and hepato-protective role, etc. The paper presents the results of some publications in the field, focusing on the plants role in the human body detox.

***Key words**: detoxification, lead, mercury, medicinal plants, morphological characters.*

INTRODUCTION

Heavy metals are found in the nature and they are essential to life, but they can become toxic through accumulation in organisms, being associated with environmental pollution, toxicity and adverse effects on biota.

Heavy metals are mostly defined in terms of high density and relative atomic mass (Kolller & Saleh, 2018).

Increasing urbanization and industrialization, mining, waste dumps, road traffic, etc., have contributed to the deterioration of the environment and depreciation of human health (Briffa et al., 2020; Yu et al., 2023).

Heavy metals' pollution has become a galloping worldwide problem that must be prioritized. Exposure to heavy metals can be done in different ways: inhalation, consume of contaminated food & water (Masindi & Muedi, 2018), skin touch and so on. As a direct consequence, several health problems may occur, such as: neurological & blood abnormalities, cardiovascular and neurological diseases, diabetes and various types of cancer. Also, heavy metals can interact with some elements, such as calcium, iron and zinc, affecting the body's normal metabolism (Lopez Alonso et al., 2004).

Since ancient times, medicinal plants have been used as remedies for various ailments, in most countries of the world, even if the active principles were not deeply known (Jamshidi-Kia et al., 2017).

Nowadays, the medicinal plants have an important place in traditional medicine or as adjuncts in classical medicine. There are numerous studies that state the pharmacological value of the medicinal plants and their role in body detoxification and alleviation of vary disorders (Luchian et al., 2017; Toma & Luchian, 2019; Toma et al., 2020; Luchian et al, 2021a; 2021b; Toma et al., 2022; Burzo and Badea, 2023).

This review aims to analyse the most aggressive and wide spread heavy metals and how they affect people's health, as well as the species of plants useful to remove or diminish them from the body, both from Romanian spontaneous flora and cultivated areas, their list not being exhaustive.

MATERIALS AND METHODS

An electronic search for literature was carried out using the following databases: PubMed, Google Scholar, Google, Scopus, Research Gate, Direct Science, Web of Science, Science Notes and Academic Journals. The present paper presents some of the most effective natural remedies in the elimination of heavy metals from the human body, knowing that their chelation treatment is difficult and with possible side effects.

The analysed papers provided pre-clinical research to those interested. In the case of antioxidants supplementation and the use of medicinal plants, the therapy with chelating agents is much more effective (Flora et al., 2008). Various studies, as those conducted by Amadi et al. (2019) and Mehrandish et al. (2019) reported mitigation of metal toxicity from whole, parts, or extracts of medicinal plants. The herbal treatments can lead to the elimination of heavy metals by intensifying diuresis, purifying the blood, through their depurative and hepato-protective activity (Algandaby et al., 2021; Sobhani et al., 2022).

RESULTS AND DISCUSSIONS

There are 35 metals under the attention of international health and environmental organizations - in order of increasing atomic number, as it's mentioned at https://science notes.org /list/ metals (In resources for authors: List of metals. (n.d.) Science Notes. Retrieved from: https://sciencenotes.org/ list-metals): lithium, beryllium, sodium, magnesium, aluminium, potassium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, strontium, zirconium, niobium, molybdenum, palladium, silver, cadmium, indium, tin, cesium, barium, tungsten, iridium, platinum, gold, mercury, thallium, lead, bismuth, radium, uranium), of which 23 are heavy metals as it is mentioned in the paper of Tchounwou et al. (2012): aluminium, antinomy, arsenic, barium, beryllium, bismuth, cadmium, gold, indium, lead, lithium, mercury, nickel, platinum, silver, strontium, tellurium, thallium, tin, titanium, vanadium, uranium and zirconium), because their specific weight (S.W.) is at least 5 times higher than that of water (S.W.=1 N/m^3). Some heavy metals, such as iron, copper, magnesium and zinc, are essential in carrying out metabolic processes, but only in accurate amounts. Among the most toxic and widespread heavy metals with a serious impact on human health are mentioned: arsenic (S.W.=5.7 N/m^3), cadmium (S.W.=8.6 N/m^3), mercury (S.W.= 13.5 N/m^3) and lead (S.W.=11.3 N/m^3). Heavy metals do not decompose themselves, being extremely difficult to be removed and, once accumulated, their concentration per unit mass increases in the body.

Arsenic (As) is an extremely toxic metalloid for human beings, coming from natural and anthropogenic sources (Lim et al., 2014), e.g. industry, pesticide manufacturing, etc. The people can be exposed to arsenic by drinking, inhaling or direct contact, etc. High As concentrations were found in the plasma and cerebrospinal fluid of patients with Alzheimer's and Parkinson's diseases (Cheng et al., 2007). Arsenic generates reactive oxygen species (ROS), which cause cell damage followed by death. For this reason, arsenic compounds were used in ancient times to treat malaria, cancer, plague, and syphilis (Khan et al., 2022). However, several studies have shown the link between arsenic exposure and the onset of certain diseases & disorders. Long-term exposure to inorganic arsenic may cause various cardiovascular disorders, such as atherosclerosis, hypertension, ischemic heart diseases and ventricular arrhythmias (Tsai et al., 2001; Balakumar & Kaur, 2009). Prolonged As exposure can decrease insulin sensitivity, responsible to induce type II diabetes (Paul et al., 2007). Other imbalances produced by arsenic could be: hearing problems, blood abnormalities, venous insufficiency, fatigue, headaches, dermatitis, salivation overflow, hair loss, nails damage, kidney & liver diseases (Hedayati, 2016) and cancer (Majumdar, 2017). Carcinogenic effects of arsenic on lungs, liver, bladder, kidneys and skin are well known (Waalkes et al., 2004). Some medicinal plants, used single or in combination with synthesis medicines, can realize a synergistic effect in the chelation process (Muthumani & Prabu, 2012; Das & Chaudhur, 2014; Gupta et al., 2015; Miltonprabu & Sumedha, 2015, Mohaddese, 2021). Various studies have been done highlighting the role of flavonoids, stilbenoids (Mishra et al., 2022) and other kinds of bio-compounds in the alleviation of arsenic toxicity (Bjorklund et al., 2020, Khan et al., 2022). Bhattacharya (2017) stated that some medicinal plants can help in the amelioration from sub-chronic As toxicity through their antioxidant properties. Thus, *Trichosanthes dioica* fruit possessed remarkable alleviative effects due to its content of quercetin and vitamin C against arsenic induced myocardial toxicity (Bhattacharya et al., 2014). The ethanolic extract of *Hippophae rhamnoides* and *Triticum aestivum* worked in the same direction based to their anti-oxidant value (Lakshmi et al., 2015). *Mentha piperita* extract showed significant alteration in lipid peroxidation and free radicals scavenging by redu-cing genotoxicity and exhibiting hepato-protective and nephro-protective effects (Sharma et al., 2007). Vineetha et al., (2014) highlighted the protective role of polyphenols found in the apple peel, in case of heart injury produced by the action of arsenic trioxide. The extract of *Vitis vinifera* seeds, protects the liver (Li et al., 2015) and *Viscum album* extracts has reduced the elevated plasma levels of liver enzymes and clastogenicity induced by sodium arsenite in rats (Adegboyega & Odunola, 2012). The *Zea mays* extracts might protect rats from accumulation of arsenic in different tissues and oxidative stress, which is reflected by the increasing the glutathione concentration in those tissues (Chowdhury et al., 2009). *Spinacia oleracea* is a rich source of antioxidants and micronutrients, which could be a good solution for the management of arsenicosis (Umar, 2007). Some bio-compounds found in garlic, mainly the allicin used in aqueous extracts, appeared to be very helpful in arsenic toxicity (Chowdhury et al., 2008).

About 13,000 tons of **cadmium (Cd)** are produced annually worldwide, mainly for nickel-cadmium batteries, pigments, chemical stabilizers, various alloys. Chronic Cd exposure suppresses serotonin and acetylcholine levels, and its inhalation triggers the development of lung cancer. Also, direct Cd contact can proliferate prostatic lesions, including adeno-carcinoma. Cadmium toxicity affects the digestive tract, kidneys, liver, lungs, pancreas, brain, testicles, urethra, heart and central nervous system. Among the diseases due to cadmium poisoning are listed: hypertension, osteoporosis, anaemia, hair loss, non-hyper-trophic emphysema, cell apoptosis (Hernández-Cruz et al., 2022; Zhang et al., 2023; Oosterwijk, 2023) which can lead to coma and even death (Satarug et al., 2003). Cadmium is also known to cause deleterious effects by deactivating DNA repairing (Genchi et al., 2020; Mc Murray & Tainer, 2003). Using garlic (*Allium sativum)* extract, some researchers demonstrated Cd reduction in rats (Sadeghi et al., 2013). Other studies showed that garlic is a successful agent of Cd chelation (Boonpeng et al., 2014) and the *Aronia melanocarpa* anthocyanins as well (Mężyńska et al., 2019; Smereczański et al., 2023). Bamidele et al. (2012) noticed that Cd toxicity effects were significantly controlled by methanolic extract of *Momordica charantia* and good results were obtained by the use of *Solanum tuberosum* extract (Lawal et al., 2011). Moreover, when coriander (*Coriandrum sativum*) was introduced as a supplement, together with garlic and *Chlorella* algae in the diets of *Prussian* carp (*Carassius gibelio*), the protection against kidney damage from Cd exposure was observed (Nicula, 2016). Essential polyphenols found in grape and apple showed a protective action in case of rat's kidney injury due to cadmium poisoning (Handan et al., 2020). The bio-compound oryzanol from *Oryza sativa* protects against acute Cd-induced oxidative damage in mice testicles (Spiazzi et al., 2013). Suliman (2017) has studied the effect of *Arctium lappa* against Cd genotoxicity and histopathology in kidney. Extract of *Nasturtium officinale* have protective effect on arsenic-induced damage of blood cells (Zargari et. al., 2015). Other scientific papers showed that Cd induced arterial and cardiac injuries could be significantly reduced by introducing soybean supplementation in the diet (Pérez Díaz et al., 2013).

Mercury (Hg) is a very toxic heavy metal which is widely dispersed in the nature (Bernhoft, 2012). Human Hg exposure is caused by its release from dental amalgam, vaccines, consumption of contaminated fish and seafood or through occupational exposure. Atmospheric Hg exposure can occur through volcanic activity, mining, etc. Elemental, inorganic and organic forms of mercury (Abdel-Salam et al.,

2018) exhibit toxicologic characteristics including neuro and nephrotoxicity (Zalups, 2000), gastro-intestinal toxicity with ulceration and haemorrhage, reduced sensory abilities, anorexia, fatigue, irritability and excitability, cardiovascular disease, hypertension, respiratory, immune and reproductive disorders. Hg toxicity also induces a number of stress proteins (Papaconstantinou et al., 2003) and can affect human health by causing severe changes in tissues (Zahir et al., 2005). Mercury leads to the formation of reactive oxygen species, causing DNA damage, but can also lead to carcinogenesis (Flora and Pachauri, 2010). Bulb extract of *Allium sativum* is very useful in blood disorders, such as the peripheral leukocytes damage (Abdalla et al., 2010) and brain damage (Bhattacharya, 2018) induced by Hg poisoning, due to the antioxidant potential, done by its high amount of polyphenols. *Rheum palmatum* (rhubarb) root extract, in particular, its anthraquinones, have been used with good results in kidney Hg damage on rats (Gao et al., 2016), showing significant declines of serum creatine & urea nitrogen values and increases of total protein albumin in treated groups. Seven days oral administration of *Eruca sativa* seeds extract highlighted an antioxidant, protective and curative renal activity on Hg-induced nephrotoxicity on rats (Alam et al., 2007). Kavitha et Jagadeesan (2006) evaluated the renal functions at mercury intoxicated rats, proving how kidney parameters reached to near normal after administration of fruit extract of *Tribulus terrestris* for seven days. The consumption of wormwood (*Artemisia absinthium)* aqueous extract during four weeks restored the enzymes activities perturbed by exposure to lead, offering a protective action against lipid peroxidation. In fact, the dried leaves and stem of wormwood were grinded in a blender with water and then centrifugated to eliminate the solid waste. The sterile aqueous extract was finally obtained by a series of filtrations and then orally administrated to rats (200 mg per every kg of body weight), as appears in the study of Kharoubi et al. (2008). Same extract showed also a protective action in brain dysfunction induced by $HgCl_2$ (Hallal et al., 2016). Abdel-Salam et al. (2018) studied the effect of dandelion (*Taraxacum officinale)* and *Coriandrum sativum*. The coriander, called, also, cilantro is a popular culinary and medicinal herb which is very recommended in heavy metals detox, mainly mercury and lead (Rafati-Rahimzadeh et al., 2017), as well as *Viola tricolor.* Their mechanism is to enhance the Hg excretion (Abascal & Yarnell, 2012), improving its clearance in a number of patients poisoned with heavy metals (Mehrandish et al., 2019). Coriander ethanolic extract is highly effective to take out the mercury stored at the brain level. *Urtica dioica* is also found to be protective at the level of different organs in case of mercury poisoning (Jaiswal et al., 2022), including the brain, liver, lungs, kidney, ovary (Siouda & Abdennour, 2015). Alfalfa (*Medicago sativa*) extract, by its nutritional and antioxidative activities, could also decrease the Hg toxicity-induced and could improve the structure and function of the kidney and liver (Raeeszadeh et al., 2021). *Allium ursinum* is indicated for binding heavy metals using sulphur compounds to eliminate them through kidneys; an important compound is the glutathione peptide which is responsible for the detoxification of free radicals and their neutralization in the human body (Sobolewska et al., 2015).

Lead (Pb) is a heavy metal used in different industrial plants and emitted from some petrol motor engines, batteries, radiators, waste incinerators, and residual water (Manisalidis et al., 2020). Lead poisoning, called saturnism (Montes-Santiago, 2013) is correlated with the bluish tinge of the skin around lips, eyes, gums and nail beds. This colour represents the sign of cyanosis. It may not appear until oxygen saturation falls below 85%. The blue colour of cyanosis means that organs, muscles and other body tissues do not receive enough amount of oxygen to operate properly. The cyanosis symptoms are due to drugs overdose or poisoning, including heavy metals toxicity, heart & lung injuries or some autoimmune diseases. The lead poisoning can generate brain damage, even in animals' liver, kidneys (Aziz et al., 2012), bones and diseases of the digestive system, central nervous system (Saleh et al., 2019), and the reproductive system (Abdou et al., 2006), causing metallic taste, weight loss and headaches, insomnia and metabolic dysregulation of vitamin D (Mutter et al., 2010). Children are extremely sensitive even to minimal doses of lead, being highly neurotoxic

(Assi et al., 2016) and it causes hearing loss, hyperactivity, aggressiveness, learning difficulties, impairment of memory (Soodi et al., 2008) and even mental retardation (Farhat et al., 2013). *Ginkgo biloba* is one of the herbal remedies used as a complementary treatment for lead-poisoned patients, by reducing the oxidative stress and elevating the glutathione level (Tunali-Akbay et al., 2007). The aqueous extract of coriander seeds was effective in normalizing the adverse effects of lead induced nephrotoxicity (El-Masry et al., 2016). Several natural products involved in nephroprotection (Sri Laasya et al., 2020), such as phenolics and flavonoids, can reduce renal damage, lipid peroxidation, urea and creatinine reduction (Gholamine et al., 2021) by decreasing the oxidative stress and protecting the liver (Kükner et al., 2021; Mahmoud et al., 2023). Other researches showed highly protective effect of *Aquilegia vulgaris* against lead acetate-induced oxidative stress in rats (El-Nekeety et al., 2009*).* Cilantro has a wide range of healing and body detox properties by removing toxic residues, such as lead, and excreting them (Tellez-Lopez et al., 2017). *Coriandrum sativum* extract has an extensive application in treating pathological situations of nervous tissues, nervous disorders affected by heavy metals toxicity (Ghosh et al., 2017). Coriander showed, also, encouraging results as chelation and poisoning reduction in animal studies, in case of lead intoxication (Velaga et al., 2014). Ghanem et al. (2008) demonstrated that *Cynara scolymus*, due to its volatile constituents is useful to fight against lead toxicity, especially at liver & kidney level. Falah (2012) has also reported that *Ficus carica* rendered hepatoprotective effects in animal studies. Waggas (2012) showed that grape seed extract (*Vitis vinifera)* alleviate neurotoxicity and hepatotoxicity induced by lead acetate in male albino rats. *Rosmarinus officinalis* was very helpful to treat the lead injuries at the level of liver and kidney (Mohamed et al., 2016). Ishiaq et al. (2011) stated that lycopene, the valuable antioxidant found in tomato fruit, may improve the enzymatic activity in case of kidney Pb damage, while Jarad (2012) stated that freshly prepared aqueous extract of *Allium sativum* alleviated the liver Pb damage. Chinthana and Ananthi (2012) have reported that the neurotoxicity induced by lead in albino mice could be significantly reduced by administration of *Solanum nigrum* extract. Ethanolic extract of *Tagetes erecta* balanced the antioxidants' level and decreased lipid peroxidation in Pb intoxicated rats (Adikwu et al., 2013). Some plants can reduce the bio-availability and gastrointestinal absorption of heavy metals by increasing gastro-intestinal motility, so that a faster excretion of toxic substances is achieved through stool. Due to this fact, the people with regular use of herbal products can significantly reduce the absorption of heavy metals (De Smet et al., 1992). In the traditional Chinese medicine, the roots of *Arctium lappa* (the burdock) are considered a blood detoxifying agent (Raeeszadeh et al., 2021). In an Egyptian study conducted by Algandaby et al. (2021) seven medicinal plants (*Arctium lappa* - roots, ratio 20:100, *Coriandrum sativum* - leaves, ratio 10:100, *Olea europaea* - leaves, ratio 15:100, *Silybum marianum* - fruits, ratio 20:100, *Tribulus terrestris* - shoots, ratio 20:100, *Urtica dioica* - shoots 15:100) were used as chelating compounds to alleviate toxicity of heavy metals. The formula was administrated six months, half an hour before meal (near 10 g of herbal mix at 200 ml boiled water once a day or half dose twice a day). The potentiality of plant extracts to mitigate heavy metal toxicity in animals and humans is attributed to their antioxidant properties of the phytochemicals present in these extracts. Bilberry (*Vaccinium mirtylus*) anti-oxidants can scavenge free radicals and chelate the metallic ions (Mazza et al., 2002). The antioxidative mechanisms of silymarin found in *Silybum marianum* include the inhibition of reactive oxygen species (ROS), direct scavenging of free radicals' actions and ion chelation (Surai, 2015), being a supporting treatment in liver curing (Gillessen and Schmidt, 2020). The red clover (*Trifolium pratense*) aids in blood purification and also help to stimulate bile production, having the ability to remove heavy metals and toxins from the body (Nelsen et al., 2002). Sorrel (*Rumex crispus* and *Rumex acetosa)* is likewise an excellent detoxifying herb that helps with the digestion of fats and gentle elimination of heavy metals (Mostafa et al., 2011). The ripe berries of *Mahonia aquifolium* are known to enhance liver functions, bile production and blood purification (He and Mu, 2015). Vary authors highlighted the powerful role in body detox from heavy metals played by the medicinal plants, such there are: *Equisetum arvense* - lead detoxification (Pant et al, 2015), *Petroselinum crispum* - cadmium neurotoxicity (Maodaa et al., 2016), *Solidago virgaurea* - general detox (Fursenco et al., 2020), as well as *Foeniculum vulgare* (Al-Snafi, 2018), *Thymus* sp. (Afonso et al., 2020), *Oregano* sp. (Velickovic et al., 2014) and *Ocimum* sp. (Cohen, 2014) having strong antioxidative and detoxifying properties.

CONCLUSIONS

This review analyses the main species of plants used to remove heavy metals from the body or to mitigate their injuries at cell level.

The mentioned species of this paper belong to Romanian spontaneous flora and cultivated area, as well.

Trifolium pratense, *Rumex* sp., *Equisetum arvense*, *Solidago virgaurea, Thymus* sp., *Oregano* sp., *Ocimum* sp. show very strong antioxidative and detoxifying properties.

To counteract arsenic poisoning, the studies recommend the use of some spontaneous and cultivated plants, such as: *Trichosanthes dioica, Hippophae rhamnoides, Triticum aestivum, Mentha piperita, Viscum album, Zea mays, Spinacia oleracea, etc.*

Cadmium toxicity can be reduced by herbal remedies based on some medicinal and cultivated plants, as there are: *Aronia melanocarpa, Momordica charantia, Solanum tuberosum, Oryza sativa, Arctium lappa, Nasturtium officinale,* etc.

Mercury poisoning can be mitigated with the help of some plants, such as: *Allium ursinum*, *Rheum palmatum*, *Eruca sativa, Tribulus terrestris*, *Artemisia absinthium, Taraxacum officinale*, *Coriandrum sativum, Urtica dioica*, *Medicago sativa*, etc.

To alleviate lead body's damage, some plants can be used as complementary treatment: *Viola tricolor, Allium sativum, Rosmarinus officinalis, Cynara scolymus*, *Ginkgo biloba, Tagetes erecta, Silybum marianum, Vaccinium mirtylus*, *Vitis vinifera,* etc.

Re-discovery of natural herbal remedies represents increasingly obvious research of modern humans, and the "return to the nature" is the act of finding internal balance and healing.

By corroborating the data of some valuable publications in the field, the current review makes a first national inventory of spontaneous and cultivated plants and of herbal remedies valid for human body detox of heavy metals.

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CITIZENS AND URBAN HORTICULTURE RELATIONSHIP AFTER COVID-19 PANDEMIC: FACTS FROM ROMANIA, GREECE AND CYPRUS

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Abstract

Cities are facing both social and environmental challenges that affect food chain, public health, and social cohesion broadly. Terms such as "Mediterranean Diet", "Urban horticulture", "Organic Farming", "Edible Landscaping" took significant position in everyday life without being sure that we could understand sufficiently their meaning. The pandemic period highlighted how useful tools these would be if we could use them correctly both in planning and development of urban green areas. Urban landscaping and especially gardening and farming connected strongly with social, economic, agricultural, nutritional, environmental, and beautifying parameters through research field in the context of a Doctoral Thesis which took place in Romania, Greece, and Cyprus with main tool a specific questionnaire. The quantitative analysis of the responses is based on a set of 302 variables and aims to elicit information with reference to the knowledge, perception, and experiences of the respondents regarding urban horticulture.
The results of the analysis proved that the opinion of the great majority of respondents from Romania, Greece and Cyprus about urban horticulture is positive, with different percentages but for the same reasons.

Key words*: environmental challenges, pandemic Covid-19, social impact, urban horticulture.*

INTRODUCTION

Following a prolonged period of economic and health crises, all social groups living in large cities require uninterrupted access to fresh and nutritious food is a widely accepted fact. (Macnea et al., 2021). In addition, studies have shown that the pandemic has had an impact on food purchasing habits, diets, and food-related behaviours and practices (Hassen et al., 2023). Thus, new initiatives and practices that existed in the past but were not continued due to lifestyle changes came to the fore. The emergence of citizen movements related to urban gardening with a common goal, are solving the problems of urbanization and economic crisis by using different ways and tools. Urban gardening through the actions of movements can contribute to solving the problems of modern societies (Maknea & Tzortzi, 2019) while it seems to be very favorable both for social inclusion and for the reduction of inequalities between the sexes, as the 65 % of urban farmers are women (Orsini et al., 2013). A study by Nicola et al. (2020) has highlighted the value of urban vegetable gardens and their potential contribution during a Pandemic. The study also identified barriers to accessing community gardens, which were further highlighted by the Covid-19 pandemic. The authors' proposal is to be making immediate use of courtyards and balconies. Research in India has yielded similar results in mitigating the effects of the food crisis that arose during the pandemic. Urban kitchen gardening practices, such as rooftop farming, have been suggested (Kaur et al., 2024). It is a fact that developing countries have been the most affected by the pandemic (Dasgupta & Robinson, 2022). Urban agriculture can be an alternative solution to address the high demand for food in urban areas (Mishra & Pattnaik, 2021). Research findings on the impact of the COVID-19 crisis in Bosnia-Herzegovina indicate potential for the development of urban agriculture. The study suggests that citizens should support the urban agriculture movement to address food supply challenges and mitigate environmental issues related to food consumption in urban areas (Nikolić et al., 2022).

MATERIALS AND METHODS

To guarantee a comprehensive approach to urban gardening, a qualitative and quantitative research methodology has been selected that concentrates on city citizens. In addition to the experimental section, which is restricted to plants, it is also essential to investigate the impact of urban gardening on society. Consequently, we created a social survey using a questionnaire that maintains the respondents' interest by providing the required information. The aim of the questionnaire was to gather information on the opinions and attitudes of urban dwellers in Romania, Greece, and Cyprus towards urban gardening and co-cultivation. The aim of the questionnaire is to collect information on the opinions and attitudes of urban dwellers in Romania, Greece and Cyprus towards urban gardening and co-cultivation. In order to ensure a comprehensive data collection, a questionnaire was designed and distributed in three languages: Romanian, Greek and English. Various 'snowball' samples were used in the study (Parker et al., 2019). The sampling unit comprised the populations of Thessaloniki, Bucharest, and Paphos. Different methods were employed to reach the urban population in each country, including Facebook, email, WhatsApp, Viber, etc. and relevant articles published on local websites. The questionnaire was distributed to a diverse range of individuals. The study included participants who read daily news, are participating in groups- such as the Union of Romanian Greeks and environmentalists- and are active citizens in everyday life. There were no specific criteria set for participant selection, as the main objective was to gather data on the general population's knowledge and opinions about urban gardening and the cultivation of edible plants in each country. Difficulties were encountered during data collection as the number of responses received was low compared to the number of questionnaires distributed, despite the distribution period being only 5 months. The factors influencing this non-response vary. In general, questionnaires that require direct contact with respondents yield the highest response rates, while internet, telephone and mail questionnaires have low response rates (Suskie, 1996).

Online surveys are a cost-effective and efficient method for questionnaire design, data collection, storage, and visualization. However, a major drawback is that many potential participants may lack the necessary computer or internet skills to complete the survey using tools such as Google Forms. Additionally, the small screen size of mobile phones can limit the duration of the questionnaire and the quality of responses to open-ended questions (Nayak & Narayan, 2019).

The research questionnaire was anonymous, which facilitated the study. It was developed based on data from the literature review and qualitative research conducted between September and December 2022. During this period, a small sample of the population (20 persons) tested the questionnaire to determine completion time and participant comprehension. This was done to avoid difficulty in understand questions.

Regarding structure, a Likert scale was not used due to evidence that online survey participants tend to choose the middle option, resulting in answers that do not reflect reality (Schwab, 2021).

The questionnaire consisted of 26 closed-ended or multiple-choice questions, including yes/no questions. The format followed a simple question-and-answer method to attract research participants.

The questionnaire comprises four sections: demographics, gardening knowledge and views on urban gardening, personal relationship with gardening, and growing in the city.

Section 1 comprises five closed questions that accept only one answer and pertain to demographics, primarily targeting urban residents.

Section 2 solicits opinions on urban gardening, which effectively promotes it. This section employs a mix of yes/no and open-ended questions to allow respondents to answer freely without being influenced or confused by suggested responses. This increases the chances of them getting a more realistic picture of their knowledge of urban gardening and plant production. In this way, the results will significantly contribute to the research objective. This section is essential as it attempts to highlight the potential knowledge gap in this area.

Section 3 is designated for urban growers exclusively and comprises of six questions. The initial question in this section is closed and categorizes the respondents into two groups: those who have participated in urban gardening at least once and those who have not had the chance to participate, have not been given the opportunity, or are simply not interested. Respondents from the first group are required to answer five specific questions, while those from the second group are asked to answer the final section, which contains general questions. Five out of the six questions in this section are multiple choice, as they are intended for respondents with adequate knowledge of the subject.

Section 4 is the final section and presents respondents' opinions on urban development. This section is open to all citizens. Three out of the 9 questions are multiple-choice and cover the advantages of urban gardening and co-culture, as well as the role of urban agriculture. The following four questions address two fundamental issues. It is evident that the pandemic has brought to light both the negative aspects of cities and some solutions related to urbanization. Of the four questions, two are closed and two are open-ended. This allows respondents to express their opinions based on their experiences during the pandemic. The penultimate question aims to gather data on city dwellers' views on the future of urban gardening. The answer structure is multiple choice, enabling the formation of a complete sentence based on the results, whether optimistic or pessimistic. The final question on the questionnaire is open-ended, allowing respondents to provide justification for their opinions if they choose to do so.

The sample labelled as 'snowflake' was chosen because the research topic concerns all urban citizens without exception. This method refers to a sampling technique in which the researcher selects a small population of individuals, which then grows and expands like a 'snowball' (Baltar & Brunet, 2012).

RESULTS AND DISCUSSIONS

The results of the questionnaires were processed using statistical software (SPSS-Statistical Package for the Social Sciences) and presented in tables and graphs. The demographic characteristics of the 301 participants are presented below.

The statistical analysis shows that 74.1% of the participants are from Romania, 23.6% are from Greece, 1.3% from Cyprus and the remaining 1% are from other countries such as the USA and Germany.

According to the responses, 57.1% of the participants identified as female, while 42.9% identified as male.

None of the participants were under the age of 20. The majority of participants (SD=1.22) were in the age groups of 40-50 (28.9%) and 50-60 (28.6%), with the lowest participation rate among those over 60 (11.2%).

The research analysed citizens' perceptions of urban gardening in three countries. The survey results indicate that 47.9% of respondents from Greece and 61.4% from Romania were women, while no female respondents were recorded from Cyprus.

The age group with the highest response rate in Romania was 40-50 years old (33.6%), while in Greece it was 50-60 years old (52.9%). In Cyprus, the participants only covered the age groups of 30-40 years (50%) and 50-60 years (50%).

The questionnaire respondents were predominantly urban residents, with 78.9% from Greece, 70.0% from Romania, and 50.0% from Cyprus. Among the Greek respondents, 50.7% reside in gardenless apartments, while 26.8% live in houses with a private yard. Similarly, in Romania, most respondents live in houses with a private garden (40.4%) or in gardenless apartments (39.0%). In Romania, the majority of respondents live in houses with a private garden (40.4%) or in apartments without a garden (39.0%). Meanwhile, the majority of Cypriots live in a house with a private yard (50.0%), or in an apartment building with (25%) or without (25%) a garden. In terms of citizens' awareness of urban gardening, 66.2% of Greek respondents reported familiarity with this activity. In Romania, 52.9% of respondents answered positively, while in Cyprus, the figure was 75.0%. Additionally, 12.7% of Greeks and 19.7% of Romanians did not provide an answer (Figure 1).

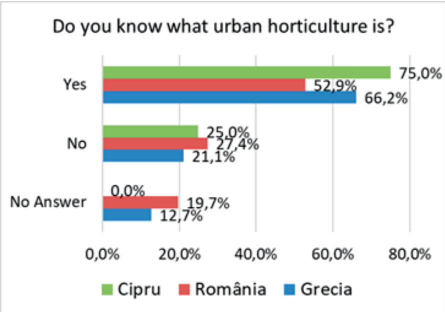

Figure 1. Results regarding the question "urban horticulture"

To gather citizens' personal opinions without being influenced by multiple-choice answers, the questionnaire included a free-response question. The responses were then categorized and quantified. The statistical analysis of the data yielded nine groups of responses, each representing the results of one country. Among Greeks, Romanians, and Cypriots, 19.7%, 8.5%, and 25%, respectively, defined urban gardening as developing in urban areas, with the highest percentage being in Cyprus. The responses to the question 'How would you define urban gardening?' can be conceptually grouped. 19.7% of Greeks and 25% of Cypriots mentioned the term 'Nutritional goal'. Additionally, 11.3% of Greeks, 16.1% of Romanians, and 25% of Cypriots mentioned 'psychological goal', 'study, learning and self-improvement goal (personal development)', 'environmental goal', 'urban biomass', 'limited space', and 'development in urban areas'. It should be noted that percentages below 10% have not been mentioned. Among respondents who were not familiar with urban gardening but provided their perception of it, only 11.3% of Greeks, 16.1% of Romanians, and 25% of Cypriots chose “growing in urban areas” as the first conceptual category. The remaining responses from these groups have percentages below 10%.

The main inquiry was whether the respondents were familiar with intercropping. The highest percentage of positive responses was recorded in Greece (49.3%), while the corresponding percentage for Romanians was 29.6% and for Cypriots, 75%. A significant percentage of Romanian respondents (50.7%) stated that they were not aware of co-culture, while 19.7% did not provide an answer (Figure 2).

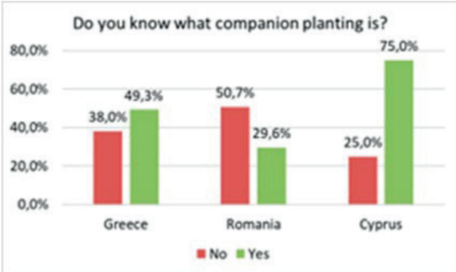

Figure 2. Results regarding the question of whether one knows what «co-cultivation» is

Participants who responded positively were asked to provide their interpretation of co-cultivation. The resulting responses were grouped into five conceptual categories to quantify the results. The category with the highest level of response was 'growing different plants in the same area', with agreement from 39.4% of Greeks, 20.2% of Romanians, and 50% of Cypriots. The four remaining categories were formed by grouping similar responses. Both the Greek and Cypriot sides received 15.5% of responses regarding 'cooperative-community gardening', with 25% of Cypriot responses in agreement. Other responses included 'combined farming with livestock' and 'biodiversity and environmental protection'. It is important to note that any unreported percentages were below 10%.

In the section on urban gardening, we asked how many city dwellers engage in cultivation. The results showed that 70.4% of Greeks, 46.4% of Romanians, and 100% of Cypriots are involved in farming. Furthermore, 14.1% of Romanians expressed an interest in learning about urban farming techniques. Those who answered positively were then asked to identify the types of plants they grow in the city. Ornamental plants were the most frequently cultivated in Greece (64.8%), Romania (57.8%), and Cyprus (100%). Aromatic medicinal plants were the secondary choice, with response rates of 34.1%, 59.2%, and 75% in Romania, Greece, and Cyprus, respectively. Vegetables were the third preferred option, with the highest percentage of choice by the Greeks (32.4%), while the Romanians showed a lower percentage of preference (17.5%). Finally, only 4.5% of the Romanian respondents confirmed that they do not engage in any form of cultivation (Figure 3).

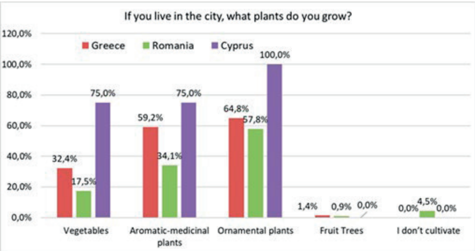

Figure 3. Results for the question about urban growers, what plants do they grow

The balcony and backyard are popular locations for growing plants, with the highest rates in Greece (64.8%) and Romania (44.4%), respectively. However, in Cyprus, growing plants on balconies is not a popular option. Only 1.4% of Greeks grow plants in their apartments, and none of the Romanians or Cypriots do. Response rates for growing plants in private and public spaces are below 10% (Figure 4).

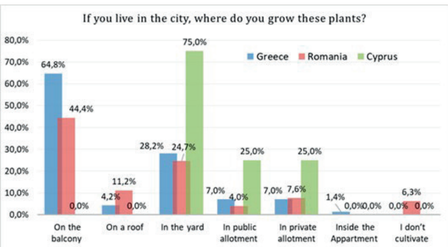

Figure 4. Results on the question about the cultivation of medicinal and aromatic plants

In an attempt to understand why aromatic medicinal plants are grown in urban areas, participants were asked to choose 5 out of 6 possible answers. The results showed that Greeks prefer these plants for their aroma (50.7%), Romanians for their appearance (39.9%), and Cypriots for their various beneficial elements (50%). In Romania, 19.3% of people do not cultivate aromatic medicinal plants, while in Greece the figure is 12.7%. In Cyprus, the percentage is lower at 4.5-5.6%, which is attributed to a lack of availability or knowledge about these plants.

In Greece, 43.7% of respondents grow each plant separately. 28.2% grow different aromatic plants together, 22.5% grow different ornamental plants together, and 12.7% grow different vegetables together. The combination of herbs and vegetables is rare in Greece, with only 1.4% of respondents using this arrangement. In Romania and Cyprus, none of the respondents reported growing herbs and vegetables together. In Romania, similar to Greece, most respondents (37.2%) cultivate each type of plant separately. Only 18.4% grow different aromatic plants together, and 21.5% grow different ornamental plants together.

The survey results indicate that relaxation is the most commonly cited benefit of urban gardening among Greeks, Romanians, and Cypriots, with rates of 74.6%, 73.5%, and 75%, respectively. The second most frequently mentioned benefit is decorating with plants that have green leaves, with percentages of 69.0% and 58.5% among Greeks and Romanians, respectively. All other benefits received lower rates (below 25%).

In terms of intercropping, both Greeks and Romanians selected the variety that provided the greatest benefit, with 52.1% and 69.5% respectively. The results indicate similarity in the choices made by the two groups, albeit with different percentages. Additionally, Greeks placed value on landscaping, while Romanians did not. All participants from different countries agreed that urban gardening has a positive impact on addressing the environmental crisis, urban development, and environmental protection. In Romania, the highest percentage of respondents (78.8%) believe that urban gardening helps educate both children and adults. Additionally, the majority of Greeks (71.8%) and all Cypriots (100.0%) share this belief.

The COVID-19 pandemic has led to an increased interest in urban gardening among respondents in Romania (80.3%), Greece

(64.3%), and Cyprus (100%). This increase is attributed to the availability of time, with 25.4% of Greeks and 36.3% of Romanians confirming this reasoning. Additionally, 50% of Cypriots surveyed attribute the increase in gardening to isolation. Romanian participants reported that relaxation and stress relief had a significant impact (31.8%) on increasing employment in urban agriculture during the pandemic.

For the widespread adoption of urban gardening, proper development of public spaces is essential. This requires a strong desire from residents. The survey collected data on citizens interested in acquiring agricultural land in their neighbourhoods. The results show that 84.8% of Romanian respondents would like to have vegetable gardens in public spaces for various reasons. These factors include psychological well-being (24.2%), access to healthy food (18.4%), environmental concerns (13.0%), socialization and education (7.6%), and aesthetic reasons (8.1%). Similarly, Greek respondents expressed a preference for vegetable gardens (87.1%) due to environmental reasons (19.7%), aesthetic reasons (16.9%), and psychological well-being (15.5%), as well as economic, social, and health reasons (11.3%). All respondents from Cyprus considered vegetable gardens necessary for aesthetic (25%) and educational (50%) purposes. Only a minority of respondents from Greece (4.2%) and Romania (4.0%) believe that creating gardens in public spaces is unfeasible. A small percentage of Greeks (2.8%) and Romanians (0.9%) perceive the environment as overpopulated. Furthermore, only a small percentage of Greeks (1.4%) and Romanians (2.7%) have access to cultivated areas for personal use. Additionally, a small percentage of Greeks (1.4%) and Romanians (0.9%) do not have time to tend to vegetable gardens. However, 66.2% of Greeks, 43% of Romanians, and 50% of Cypriots believe that the local government should establish an institutional framework for vegetable gardens. According to the survey, 49.3% of Greeks, 46.2% of Romanians, and 25.0% of Cypriots believe that the local government should create organized, social, peri-urban vegetable gardens. Additionally, 59.2% of Greeks, 54.3% of Romanians, and 50.0% of Cypriots believe that vegetable gardens should be created around residential complexes.

The survey results indicate that a majority of Greeks (74.6%), Romanians (65.9%), and Cypriots (75.0%) believe that urban gardening should be included in municipal urban planning. Additionally, a significant percentage of Greeks (49.3%), Romanians (45.3%), and Cypriots (75.0%) prefer renting private spaces for urban gardening. Meanwhile, the majority of Greeks (56.3%), Romanians (52.0%), and half of Cypriots (50.0%) prefer using public state spaces.

The study found that a majority of Greeks (74.1%) and Romanians (81.1%) are optimistic about the future of urban gardening, while only 33.3% of Cypriots share this sentiment. Additionally, a minority of Greeks (16.7%) and Romanians (12.6%) believe that urban gardening could have a future under certain conditions, compared to a majority of Cypriots (75.0%).

Concerning the future of urban gardening, the highest percentages of opinion indicate that it benefits human psychology (16.9% of Greeks and 15.2% of Romanians), the environment (15.5% of Greeks and 17.5% of Romanians), financial issues (14.1% of Greeks and 3.1% of Romanians), and dealing with a food crisis (7.0% of Greeks, 8.1% of Romanians and 25.0% of Cypriots).

Finally, 7.0% of Greek respondents and 5.4% of Romanian respondents believe that government support is essential for the future of sustainable horticulture.

CONCLUSIONS

This experiment aimed to investigate the use of intercropping in urban gardening as an environmentally friendly management plan to promote diversity. A total of 302 residents from Greece, Romania, and Cyprus participated. However, the conclusions drawn from the results in Cyprus cannot be considered representative due to the low participation rate (1.3%) and the absence of female participants. In Greece, the majority of participants are men aged between 50-60 years old, whereas in Romania, the majority are women aged between 40-50 years old. The participants are predominantly city dwellers, with almost all living in either apartments without a garden (39-50%) or houses with a private yard (26-40%).

Although most participants claim to understand the concept of urban gardening, their interpretations do not significantly differ. The general consensus is that urban gardening is associated with limited spaces, experiences, environment, education, self-improvement, psychology, and nutrition. However, there are differing opinions regarding co-culture. The third part of the questionnaire reveals that a significant proportion of residents in all participating countries cultivate plants in urban areas. A comparison of the percentages of urban dwellers and urban growers shows that while 70.9% of Romanians live in urban areas, only 46.4% grow plants. The corresponding percentages among Greeks do not differ significantly nor among Cypriots. The results indicate that participants who cultivate in Romanian cities are familiar with the concept of urban gardening, while those in Greek and Cypriot cities are not. Additionally, it was found that urban growers in all participating countries prefer ornamental plants, followed by aromatic medicinal plants and then vegetables. Based on the response rates, Greeks show a 4% difference between their first and second preferences. After comparing data of those who live in apartments (with or without a garden), it can be concluded that all Greeks who live in apartments grow plants on their balconies, while 10% fewer Romanians who live in apartments do the same. Additionally, Cypriots who live in a house with a yard use it for their crops. Although aromatic herbs are not the most popular, they remain popular in Greece due to their taste, nutritional value, and aesthetic appeal when used in tea. Accordingly, in Cyprus, these plants are considered beneficial and beautiful and are often used for making tea. In Romania, the majority of participants choose them mainly for their appearance, with their nutritional properties and taste being secondary considerations.

The study confirms the purpose of this work by demonstrating how the basic concepts of urban gardening are included in the organization of planting and cultivation. It can be concluded that the method of organizing monoculture in each pot is used more in all countries. Only 1.4% of the Greek participants co-cultivate aromatic herbs with vegetables.

Finally, the study explores the social and economic aspects of urban gardening. The aim of this project is to expand citizens' understanding of the Mediterranean diet, urban gardening, and how they can improve their quality of life in the Balkan and Mediterranean regions. This will be achieved by exploring the correlation between these topics and the practices of monasteries (Thymakis, 2022). The text discusses the possibility of including historical and botanical gardens in a type of garden based on the ecology of the rural landscape using traditional varieties, such as the 'Greek Garden' model (Thymakis & Tzortzi, 2021), as explored by Athanasiadou & Thymakis (2019) and Thymakis (2023). The survey results indicate that urban gardening is believed to contribute to education, development, and environmental protection by the majority of participants. Additionally, it offers benefits such as relaxation, decoration, and nutritional security. Similar conclusions were obtained from a corresponding study in Italy, with the same sample size. During the pandemic, 32.3% of residents engaged in urban gardening, stating that this activity correlated with a reduction in psychopathological discomfort caused by COVID-19 (Theodorou et al., 2021). In another survey, 52.24% of respondents reported high levels of satisfaction with their lives due to their engagement in urban gardening (Harding et al., 2022).

It can be concluded that employment rates in Urban Agriculture increased during the pandemic in all participating countries due to the availability of free time and psychological factors. Participants from all countries shared the desire to create open spaces near their homes to grow edible plants for various reasons, including psychological, health, environmental, aesthetic, and economic factors. Those who choose not to create a vegetable garden may find it impossible to implement, lack access to private space, or have concerns about pollution. The involvement of each Municipality and Prefecture is widely accepted as necessary for achieving these vegetable gardens, with their inclusion in urban planning. A notable aspect is the consensus among all participants on the critical role that local government should play. Both Greeks and Romanians expressed optimism about the future of urban gardening and its potential to positively impact human mental health and the environment. The Greeks also emphasized the economic benefits of urban gardening.

In conclusion, the analysis found that a large majority of respondents from Romania, Greece, and Cyprus have a positive opinion of urban gardening. Although the percentages differ, the reasons are the same. The research aims to interpret the perceptions of each group represented by a specific number of participants. However, the questionnaire did not receive the expected number of responses, so the conclusions can only be considered indicative. The responses to the open-ended questions indicate that urban gardening is a multifaceted and varied topic, which supports the initial hypothesis of the paper.

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THE INFLUENCE OF TOMATO ROOT EXUDATES ON STRUCTURE AND DIVERSITY OF RHIZOSPHERE COMMUNITIES OF BACTERIA AND FUNGI

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Abstract

The paper aimed to present the results of research carried out on soil microbiota as compared to that colonizing rhizosphere of tomato plants (Solanum lycopersicum L.), FLAVIOLA variety, for assessing the influence of root exudates on composition and abundance of microbial communities in relationship with benefits for plant nutrition and health conferred by their interaction. Cosmopolitan fungal species from genera Fusarium, Penicillium and Humicola, as well as bacteria from genera Bacillus, Pseudomonas, associated with Actinomycetes dominated microbial communities from soil. Rhizosphere communities were dominated by fungal species belonging to genera Aspergillus, Penicillium and Trichoderma accompanied by nematophagous fungi from genus Arthrobotrys. Pseudomonas fluorescens, important for biocontrol of root pathogenic microorganisms, associated with other non-fluorescent species of Pseudomonas were the most abundant in rhizosphere. Rhizosphere effect assessed using as indicator the ratio between the value of total counts of microbes in rhizosphere and in soil (R/S) confirmed the stimulation of microbial abundance and diversity by plant root exudates as compared to soil.

Key words: *rhizosphere effect, tomato roots, biocontrol agents, microbial communities, root exudates.*

INTRODUCTION

Soil is a complex system of habitats providing variable conditions and hosting a microbial biodiversity complex with multiple microbe-microbe, microbe-environment and plant-microbe, interactions (Prasad et al., 2021). Plants rhizosphere is a place different from bulk soil because it is enriched in various metabolic compounds permanently released by roots (Verma et al., 2018). Their composition is variable in time during the development stages of plant and other environment conditions (pH, temperature, light) (Singh et al., 2007). These exudates consist in carbon-based compounds (organic acids, amino acids, sugars, enzymes, mucilage) playing the role of attractants for various species of microorganisms belonging to the groups of bacteria and fungi (Reinhold-Hurek et al., 2015).

Creighton Miller et al. (1986) and Rasmann & Turlings (2016) stated that these root signals are also able to mediate mutualistic interactions in the rhizosphere. Research carried out by Fu et al. (2021) revealed that differences in bacterial and fungal community composition correlated with specific modifications in composition of root exudates released by tomato plants across different developmental stages. Rhizosphere microbiota, protected by microenvironment conditions from structural aggregates presents a more intense activity, releasing also various metabolic biosynthesized products: enzymes, exopolysaccharides with role in binding soil particles, pigments, compounds with structure similar to humic substances belonging to aromatic group including quinones, anthraquinones, naphthoquinones, flavines, proteins (Matei et al., 2022). ACC deaminase produced by plant-growth promoting rhizobacteria was able to alleviate salinity stress in French bean (*Phaseolus vulgaris* L.) plants (Gupta & Pandey, 2019) and improved drought stress tolerance in grapevine (*Vitis vinifera* L.) (Duan et al., 2021).

A recent study using modern approach revealed that microbial carbon use efficiency (CUE) is a major determinant of soil organic carbon (SOC) storage, supporting the idea that a high microbial CUE promotes SOC storage more than SOC loss. The high efficiency leads to

SOC accumulation by increased synthesis of microbial biomass and by-products but a low efficiency drives to SOC loss by partitioning more carbon towards cellular respiration (Tao et al., 2023).

Thus, plants rhizosphere and their bacteriobiome and mycobiome play an important role in carbon (C) sequestration and combating the negative effects of climate changes.

Microbial necromass is considered as the main component of SOC sequestration, its contribution to aggregate-C sequestration being of 43.96% with higher values from fungal necromass-C than that of bacterial (Zhang et al., 2023).

Research has been carried out on soil microbiota as compared to that colonizing rhizosphere of tomato plants, for assessing the influence of root exudates on composition and abundance of microbial communities in relationship with benefits for plant nutrition and health conferred by their interaction.

MATERIALS AND METHODS

In order to assess the influence of root exudates of plants on composition and abundance of microbial communities an experiment was conducted in Mitscherlich pots with 8 kg soil Typic Chernozem (WRB) from Fundulea (with pH 7.51, humus content 3.18%, N content 0.154%, C/N 14, PAL 38ppm, KAL 163 ppm) with tomato plants grown under control conditions at NRDISSAE, Bucharest, during the summer 2023.

Romanian cultivar FLAVIOLA is characterized by high foliar biomass, small, uniform and very tasty tomato fruits, with a high productivity on various culture substrates or greenhouse technologies (Drăghici & Pele, 2012; Jerca et al., 2016; Jerca et al., 2021; Matei et al., 2023).

After 60 days, soil and rhizosphere samples were collected and analysed according to the specific methods of chemical and microbiological analysis described in the manual utilized in soil monitoring system (Dumitru & Manea, 2011).

Thus, microbiological parameters determined for soil and rhizosphere by plating serial dilution on solid agar culture media, were: Total Number of Bacteria-TNB (on Nutrient agar - NA) and Total Number of Fungi-TNF (on potato-dextrose agar - PDA), taxonomic composition of microbiota, carried out by morphologic criteria (using a MC5.A optic microscope) and specific manuals for bacteria (Bergey & Holt, 1994) and for fungi (Cooke & Godfrey, 1964; Domsch & Gams, 1970; Liu et al., 1992; Dackman, 1992; Watanabe, 2002).

Rhizosphere effect was assessed using as indicator the ratio between the value of total counts of microbes in rhizosphere and in bulk soil (R/S).

Another parameter represented by the global physiological activity of microbiota (expressed as the quantity of CO_2 released by soil, respectively rhizosphere microbial communities) was assessed by substrate induced respiration (SIR) method and bacterial and fungal biomass calculation.

According to this selective inhibition technique (Bailey et al., 2002), soil and rhizosphere microbial community structure was established by interpreting the values of the ratio of fungal to bacterial biomass (F: B).

The species richness or total number of species (S), as well as the relative abundance (A%) of each species in microbial communities from soil and tomato rhizosphere were calculated.

Microbial biodiversity in bulk soil and in rhizosphere of tomato plants was also assessed by calculating the diversity index Simpson (D) and Shannon index (H′), its value taking into account both the richness and evenness E(ε) of the community (Mohan & Ardelean, 1993).

RESULTS AND DISCUSSIONS

The rhizosphere of tomato plants is represented by soil aggregates adhering to the roots, formed by soil particles, bound together with decomposed organic residues, root exudates (rich in phenolics) and exometabolites released by microorganisms (Figure 1), as noticed in other research (Makoil & Ndakidemi, 2007), revealing the composition of exudates binding aggregates in rhizosphere of leguminous plants and their role in providing support for plant roots.

Allard et al. (2016) reported that conditions from *Solanum lycopersicum* (tomato) rhizosphere influenced the composition and structure of robust bacterial communities when grown in soil amended with various organic and synthetic fertilizers.

Figure 1. Soil aggregates in rhizosphere of tomato plants

Arshad et al. (2023), Jerca et al. (2023) underlined the importance of climatic conditions from the greenhouse on tomato production and fruits quality.

Analysis of results presented in Table 1 reflects the general tendency of increasing the total counts of both groups of microorganisms with one order of magnitude under the influence of root exudates released by tomato plants as compared with microbiota from bulk soil.

Thus, total counts of heterotrophic aerobic bacteria increased from low value of 9.974 x 10^6 viable cells x g^{-1} dry soil for bulk soil to high value of 38.372 x 10^6 viable cells x g^{-1} dry soil in rhizosphere and from moderate value of 89.448 x 10^3 colony forming units (c.f.us) x g^{-1} dry soil to high value of 136.415 x 10^3 colony forming units x g^{-1} dry soil for microscopic fungi.

Table 1. The total counts of bacteria, fungi in soil and rhizosphere microbiota and R/S index

Origin of microbiota	TNB (x 10^6 viable cells x g^{-1} d.s.)	R/S	TNF (x 10^3 cfus x g^{-1} d.s.)	R/S
Soil microbiota	9.974	3.847	89.448	1.525
Rhizosphere microbiota	38.372		136. 415	

The value of R/S ratio (3.847) for bacteria, higher than the value calculated for fungi (1.525) indicates that the composition of root exudates released by tomato plants was very appropriate and stimulated the development of a numerous bacterial community and to a less extend for the group of fungi.

The species *Bacillus megaterium* (A=22.2%) was dominant in composition of soil bacteriobiome, accompanied by other species of the genus *Pseudomonas*, *Bacillus*, *Arthrobacter* and actinomycetes from Series Albus, Fuscus and Luteus (Figure 2).

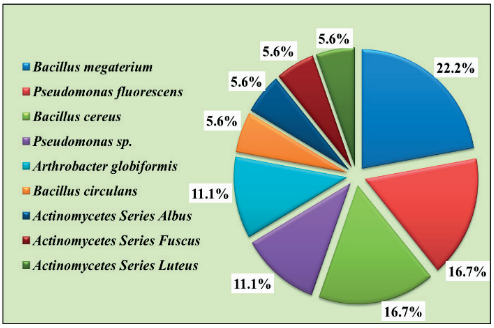

Figure 2. Composition and species relative abundance in soil bacteriobiome

A higher number of species (17) influenced by the presence of tomato root exudates has developed in rhizosphere conditions (Figure 3).

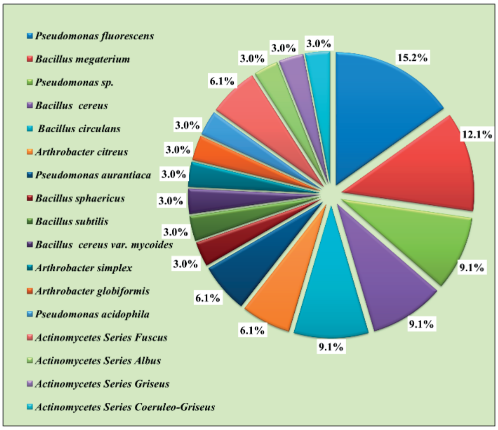

Figure 3. Composition and species relative abundance in rhizosphere bacteriobiome

The most abundant species was *Pseudomonas fluorescens* (A=15.2%), recognized for the role of biocontrol agent of plant pathogens, accompanied by a larger number of species from genera *Pseudomonas, Bacillus, Arthrobacter* and actinomycetes from Series Fuscus, Albus, Griseus and Coeruleo-Griseus.

Fungal community (mycobiome) from soil consists of 9 species, dominated by *Penicillium aurantiogriseum* (A=21.4%), accompanied by other 3 cosmopolitan species from genera *Aspergillus, Fusarium* and *Humicola* (each with A=14.3%) and 5 species (A=7.1% each) with high cellulolytic capacities, too (Figure 4).

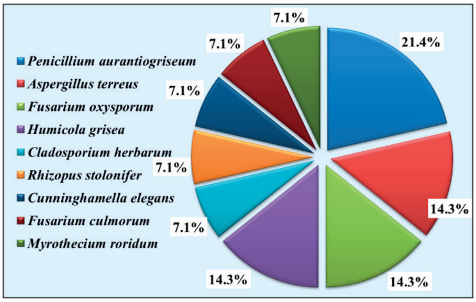

Figure 4. Composition and species relative abundance in soil mycobiome

Conditions created in rhizosphere by exudates excreted by tomato roots sustained a more complex mycobiome consisting of 13 species (Figure 5), with 5 of them twice more abundant (each with A=11.11%) than the other 8 species (each with A=5.6%). The five codominant species were antagonists of phytopathogens (*Trichoderma viride*), strong cellulose decomposers from genera *Penicillium* and *Aspergillus* and nematophagous fungus *Arthrobotrys arthrobotryoides*.

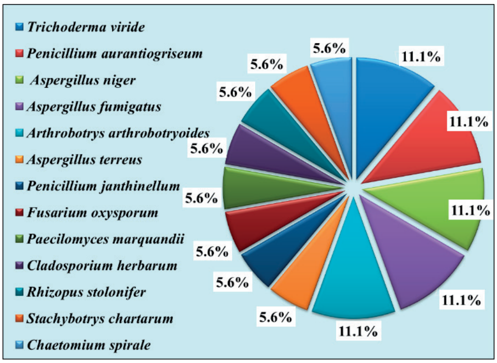

Figure 5. Composition and species relative abundance in rhizosphere mycobiome

Figure 6 illustrates the capacity of these fungal species to produce exometabolites represented by surface yellow or uncoloured exudates (from species of *Penicillium* and *Aspergillus*) and red pigments diffused into the medium as in the case of interactions between species from genera *Trichoderma, Penicillium* and *Fusarium*.

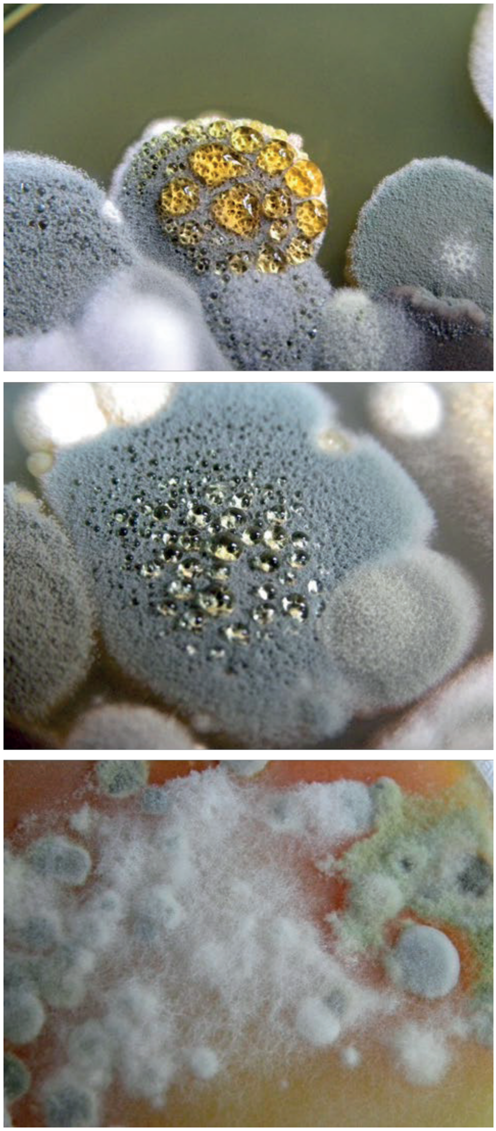

Figure 6. Fungal exudates and pigments produced by species from rhizosphere mycobiome

Results from present research are in concordance with those reported and reviewed by Abdul-Rahman et al. (2021) who found that root exudates accumulated by "rhizodeposition" in rhizosphere ecosystem improved conditions for increasing the density and biomass accumulation of favourable bacteria, as well as higher rhizosphere respiration rates compared with surface soils. The prevalence of pseudomonads, bacilli and actinomycetes in the rhizosphere of tomato plants complies with the results of other studies on tomato rhizosphere, soil and roots evidencing the presence of Proteobacteria, Actinobacteria, symbiotic and free living diazotrophic microbes, mycorrhizal fungi and phosphate solubilizing microorganisms in composition of rhizosphere microbiome, with importance in biogeochemical cycles of nitrogen, carbon, phosphorus and sulphur (Kalayu, 2019; Abdul Rahman et al., 2021; Trivedi et al., 2021; Anzalone et al., 2022; Naumova et al., 2022).

Alawiye & Babalola (2019) remarked the high biodiversity in bacterial communities from typical rhizosphere of plants and discussed the interactions and their importance for both microbes and plants.

The higher number of fungi in rhizosphere compared to soil has a beneficial effect on C sequestration in their cell walls and enhance C stabilisation by facilitating soil aggregation.

Other researchers (Six et al., 2006) stressed the importance of both groups of microorganisms, interdependent each other, and their role in the main processes from soil and rhizosphere in agroecosystems.

The rhizosphere effect was evidenced by higher values of diversity indices registered for both bacterial and fungal communities as compared with those characterizing the communities from bulk soil (Table 2).

Table 2. Bacterial and fungal biodiversity in soil and rhizosphere microbiota

Origin of microbiota	Bacterial Diversity	Fungal Diversity
Soil microbiota	S=9 Simpson D=0.858 Shannon H′=2.062bit E(ε)=0.837	S=9 Simpson D=0.867 Shannon H′=2.107bit E(ε)=0.829
Rhizosphere microbiota	S=17 Simpson D=0.918 Shannon H′=2.659bit E(ε)=0.853	S=13 Simpson D=0.904 Shannon H′=2.505bit E(ε)=0.847

The communities from rhizosphere were richer in species and more homogenous concerning the distribution of "individuals" on species, as reflected by the increased values of E(ε)=0.853 for bacteria and E(ε)=0.847 for fungi, comparatively with lower values for soil microbiota. These data confirmed the more homogenous values of abundance (A%) of species from rhizosphere communities previously illustrated.

Similarly, results of Naumova et al. (2022) on bacterial species richness revealed values with an order of magnitude higher in the rhizosphere than in the root endosphere and were explained by the higher versatility of environmental niches in the rhizosphere. The results in the present research are in concordance with a very recent study (Alahmad et al., 2024) concerning the composition of microbial communities and interactions under 4 pearl millet (PM) lines with various potentials of soil aggregating. The rhizosheath, or root-adhering soil (RAS), defined as the cohesive soil layer firmly adhering to plant roots (with role in facilitating uptake of water and nutrients) presented distinct microbial community as compared with those colonizing root tissue (RT) and bulk soil (BS), for both bacteria and fungi. Bacterial α diversity indices (richness and evenness) were not significantly different among PM lines within compartments, but fungal diversity from RT presented much higher evenness compared to BS and RAS.

Bacterial and fungal communities from rhizosphere of tomato plants in present experiment accumulated higher biomass C than the communities from soil (Table 3). F: B ratio calculated for soil microbiota was 0.928 and 0.814 for rhizosphere microbiota. This indicated bacterial-dominated microbial community structure for both soil and rhizosphere.

Table 3. Bacterial biomass, fungal biomass and F: B ratio in soil and rhizosphere microbiota

Origin of microbiota	Bacterial biomass (μg C/g sol)	Fungal biomass (μg C/g sol)	F: B ratio
Soil microbiota	428.192	397.478	0.928
Rhizosphere microbiota	614.958	501.032	0.814

According to Wang et al. (2019), the values of F:B biomass ratios <1 reflect a bacterial-dominated microbial community. The authors argue the importance of this indicator of microbial community structure and its significance in soil ecology. Bacterial versus fungal dominance is clearly related to plant productivity. Also, a higher F: B ratio indicate a fungal-dominated microbial community and a high sustainability in agro-ecosystems.

Bailey et al., (2002) reported the value of 0.85 for F: B ratio in agricultural soil cultivated with corn and 13.5 for restored prairie soil, the last high value due to increased fungal activity being associated with increased soil C.

Results of a recent study in France (Djemiel et al., 2023) proved the usefulness of F: B ratio as an indicator of soil status, the values determined ranging from 0.24 to 12.5 being influenced by soil characteristics (especially the pH, organic C content, C:N ratio) and land management. 5.5% of soils with ratios <1 have been anthropized soils (under grasslands and various agricultural crops).

Rhizosphere microorganisms play an important role by plant growth promoting under abiotic stress conditions.

Chaudhary et al. (2018) found the beneficial role of *Penicillium* in increasing plants resistance to various abiotic stresses. Thus, various effects of several microbial species from rhizosphere were reported on cultivated tomato plants.

In drought conditions, *Pseudomonas* sp. improved antioxidant enzymes activity, increased tomato biomass accumulation, altered ABA and indole-3-acetic acid (IAA) content (Brilli et al., 2019). *Trichoderma* sp. promoted tomato plant growth and its tolerance to water deficit (through increased N and P uptake), increased shoot dry weight and stomatal conductance (Khoshmanzar et al., 2020).

Yan & Khan (2021) attributed the biological control of bacterial wilt of tomato plants to the metabolites with inhibitory role released by fungus *Trichoderma harzianum* and Mukerjee et al. (2022) evidenced the mycoparasitism as responsible for *Trichoderma*-mediated suppression of plant pathogenic agents.

Trichoderma harzianum induced an increased synthesis of secondary metabolites and defence-related enzymes by tomato (*Solanum lycopersicum*) plants as mechanisms for the control of root-knot nematodes (Yan et al., 2021). Results reviewed by Yao et al. (2023) confirmed the role of *Trichoderma* in biological control of fungal pathogens and nematodes by competition, antagonism, antibiosis and mycoparasitism and in promoting plant growth.

Yoo et al. (2021) reported that tomato plants presented increased tolerance to salinity stress induced by 2 strains of bacteria belonging to the genus *Bacillus* that increased proline accumulation, antioxidant enzymes activity, chlorophyll and carotenoid content and prevented the damage of plant cell membrane.

Other authors (Calderón et al., 2014) evidenced the role of 2-hexyl, 5-propyl resorcinol produced by a strain of *Pseudomonas chlororaphis* in the multitrophic interactions from rhizosphere of avocado during the biocontrol processes.

Recent results (Boiu-Sicuia et al., 2023) from a study carried out for the evaluation of antifungal potential of 6 bacterial strains belonging to *Bacillus pumilus*, *B. subtilis* and *B. velezensis* isolated from various plants revealed the broad and strong antifungal effect of both living cells, bacterial cell-free supernatants and volatile active compounds on various species of grape spoilage fungi (from genera *Aspergillus, Penicillium, Botrytis*) and grapevine trunk disease fungi (from genera *Fusarium, Diplodia, Clonostachys, Neofuscicoccum, Stereum* and *Eutypa*).

Some strains were able to produce fungal growth inhibition by synthesis of lytic enzymes (chitinase, cellulase, protease), difficidin, fengycin, iturins, macrolactin and mycosubtilin. Also, plant beneficial effects were found (e.g. phosphate and phytate solubilization, phytohormone synthesis).

Diazotrophic/N-scavenging bacteria isolated from the soils and rhizospheres of two species of *Solanum* presented plant growth-promoting activity (Zuluaga et al., 2020).

The symbiosis between vesicular-arbuscular mycorrhiza (VAM) in some Nigerian soils and three cultivated plants improved the growth of cowpea (*Vigna unguiculata*), tomato (*Lyccopersicon esculentum*) and maize (*Zea mays*) (Sanni, 1976).

Research on the interactions between VAM fungi and rhizosphere microbiota revealed, as compared with non-mycorrhizal gramineous plants, changes in frequency of certain species, such as decrease of % of phytopathogens from genera *Fusarium, Helminthosporium, Alternaria, Pythium* and increasing the representation of beneficial cellulolytic species from genera *Cladosporium, Scopulariopsis*, nematophagous fungi and *Trichoderma*, the last being involved in pathogens inhibition by volatile compounds released (Șesan et al., 2010; Matei, 2011), similarly to results reported by Pescador et al. (2022).

One of the first volatile compound descovered was 6-pentyl-α-pyrone, characterized by a distinct coconut odour, specific to *Trichoderma viride* which inhibited the oospore formation in *Phytophthora cinnamomi* and conidial germination in *Botrytis cinerea* (Dennis & Webster, 1971).

Actinomycetes are well-known for their capability to synthetize bioactive molecules called antibiotics, metabolites with antimicrobial role (Augustine et al., 2008), sort into major structural classes: amino glycosides, ansamycins, anthracyclines, b-lactam, macrolides and tetracyclins, more than 60% being produced by Streptomyces griseus. From 12,000 antibiotics discovered in the last 60 years, actinomycetes yielded around 70 %, the rest of 30% being produced by filamentous fungi and non-actinomycete bacteria (Rasche et al., 2006; Singh et al., 2012).

Numerous studies reported the ubiquitous presence of *Trichoderma* spp. in both soil and plant rhizosphere (Matei & Matei, 2002; Matei & Matei, 2010; Sharma & Singh, 2014) in all climatic conditions (Ghorbanpour et al., 2018) and discussed its complex interaction with plants and other microorganisms from rhizosphere or soil (Tyskiewicz et al., 2022). Alexandru et al. (2013) reported the beneficial effect of some *Trichoderma* species on photosynthesis intensity and synthesis of pigments in tomatoes.

El-Maraghy et al. (2021) evidenced the importance of the large variety of mechanisms utilized by plant growth promoting fungi, including *Trichoderma*. These fungi are also capable to synthesize siderophores of hydroxamate-type, (e.g. ferrichromes, coprogens, and fusarinines) and to suppress phytopathogenic agents by depletion of iron sources or by volatile compounds released (Harman et al., 2004; Matarese et al., 2012; Joo & Hussein, 2022).

Similarity indices between soil and tomato rhizosphere communities in present experiment had the value SI=61.53% for bacteria and SI=45.45% for fungi.

Previous results (Matei et al., 2022) evidenced that specific composition of root exudates from tomato and cucumber plants grown on similar substrates influenced the composition of rhizosphere communities of micro-mycetes, with only 52.17% shared species, half of them being nematophagous species *Arthrobotrys oligospora, Monacrosporium cionopagum* and *Harposporium anguillulae*.

Other studies (Hanson & Howell, 2004; Matei et al., 2010; Pršić & Ongena, 2020) evidenced the role of microbial elicitors produced by rhizosphere bacteria (*Pseudomonas fluorescens*) and fungi (*Trichoderma viride, T. harzianum*) in triggering plant immunity.

Kravchenko et al. (2003) noticed the effect of root exometabolites of tomato plants on the growth and antifungal activity of plant growth–promoting *Pseudomonas* strains and showed that the antifungal activity of plant growth–promoting rhizobacteria in the plant rhizosphere may depend on the sugar and organic acid (malic acid, citric, succinic acid and fumaric acid) composition of root exudates.

Our results also agree with the results of Rini & Sulochana (2007), confirming the antifungal effect of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato plants.

Results reviewed by Santoyo et al. (2012) and Boruah & Kumar (2002) evidenced the presence of edaphic species from genera *Pseudomonas* and *Bacillus* acting as biocontrol and plant growth promoting agents and explained their mechanism of action.

Veliz et al. (2017) evidenced the role of chitinase-producing bacteria in biocontrol activity.

The secondary metabolite Peptaibol Trichogin GA IV released by *Trichoderma longibrachiatum* was able to present antimicrobial activity against phytopathogenic Gram (–) bacteria from genera *Xanthomonas, Pseudomonas, Ralstonia, Agrobacterium, Erwinia* and Gram (+) *Bacillus subtlis* of cauliflower and other crucifers (Caracciolo et al., 2023).

Preece & Penuelas (2016) noticed that rhizodeposition modulates composition and structure of soil microbial communities with beneficial consequences for ecosystem resilience under drought climatic conditions.

A rich and dynamic microbiota characterized as low functional diversified may present difficulties in adapting to unfavourable environmental conditions, but rhizosphere microbiota, with high taxonomic and functional diversity is more resilient to climatic changes.
Complex interactions root-soil-microbiome in rhizosphere are important for crop production (Zhang et al., 2017), many beneficial microbes from rhizosphere being selected for use as bio-fertilizers in improving plant biomass accumulation (Thenmozhi et al., 2010).
Al-Surhanee (2022) reported improved induced resistance of tomato plants to *Fusarium* attack by using eco-friendly agents represented by *Trichoderma* and salicylic acid.
Other researchers noticed that better understanding of the biotic and abiotic factors that influence the rhizosphere microbiome composition and biomass will be important factors for selecting the most beneficial microbiome structure for enhancing agricultural productivity and ecosystem functions (Wu et al., 2023).

CONCLUSIONS

The composition of root exudates released by tomato plants was very appropriate for development of a numerous bacterial community and, to a less extend, for the group of fungi, compared to bulk soil.
The rhizosphere effect was evidenced by higher values of diversity indices registered for both bacterial and fungal communities as compared with those characterizing the communities from soil.
The ratio F:B reflected the main processes such as: nutrient cycles, decomposition, C-sequestration, self-regulation in ecosystem.
The lower value of F: B ratio in tomato rhizosphere denoted a higher importance of bacteria-dominated microbiota, with more dynamic turn -over of readily decomposable substrate and elevated microbiological activity as compared to that developed in soil.
Root exudates from rhizosphere stimulated the development of beneficial species of bacteria and fungi which can be further isolated and utilized as biological control agents, biofertilizers and organic matter decomposers in biotechnologies for plant protection, yield increasing and increasing C-sequestration for limiting the effects of climatic changes.

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THE INFLUENCE OF THE SYNTHETIC MICROBIOMES ON THE CHARACTERISTICS OF BIODIVERSITY AND CARBON SEQUESTRATION IN THE SOIL

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Abstract

The aim of the research was to evaluate the role of the microbial composition of synthetic microbiomes for soil carbon evolution. The microbiomes inoculated into the soil (M1-M9) release exometabolites that influence the transport of nutrients in the soil and the dynamics of the energy potential. Thus, after the inoculation of microbiomes in the soil, the biosynthesized metabolites, the influence on some biological parameters and on the dissolved organic carbon, released after 60 days in the soil, were analyzed. Bacterial microflora growth rate of up to 54% was determined when using M4 and M5 microbiome. Microbiomes M1 and M5 containing bacteria with antagonistic characteristics (Bacillus sp.) and hyperparasitic fungi (Trichoderma sp.) caused up to 32% increases in the microflora. Biomass induced by microbiome M4 reached values up to 354mg C x kg^{-1} soil. Microflora from each microbiome influenced differently the distribution of the fluorescent dissolved organic carbon from soil fulvic subfraction.

Key words: *microbiome; exometabolites; microbial biomass; fulvic acids; biodiversity.*

INTRODUCTION

Uptake of carbon-containing substances from the atmosphere and soil sequestration by the microbial community play an important role in the C cycle, and their activity is considered essential in determining differences in soil C storage potential. Also, soil microbial community composition is crucial for the maintenance of ecosystem services, as their structure and activity regulate nutrient delivery and organic matter decomposition rates.

The nature of soil microbiome composition is defining for soil carbon evolution. By making interventions in interactions between processes such as rates of synthesis/decomposition of organic compounds with carbon, soil carbon storage and microbiome composition, the carbon cycle (C) can be influenced. Also, the extracellular microbial content of microbiomes constitutes a competitive advantage through which they can intervene in influencing the transport of nutrients in the soil, in the dynamics of the energy potential and in the manifestation of tolerance in conditions of climatic stress.

The synthetic microbiome can also intervene in protecting/extending the activity of soil processes, stimulating the qualitative-quantitative evolution of organic carbon, facilitating microbial mobility as well as ensuring the stability of soil aggregates. Microorganisms in the composition of the microbiome function metabolically different and, in this way, directly influence the evolution of processes in the soil. Microbiomes also play an important role in nutrient cycling, ecosystem functioning, ensuring soil fertility and in plant growth development. The use and evaluation of synthetic microbiomes in terms of the impact on carbon sequestration also frames the concept of sustainable improvement of soil management, by using methods that stimulate carbon sequestration, making changes in the composition of communities, inducing structural changes in organic matter (OM) and soil aggregates, as a result of the inter- and intraspecific relationships that are established.

Synthetic microbial communities are a way to preserve the characteristics of their natural counterparts and act as model systems based on interaction/function for controlled assessments of the role of ecological, structural, and functional characteristics of communities. Also, these communities depend on the degree of microorganism dispersal, environmental selection and species sorting, with effects difficult to control or characterize (Leibold et al., 2004). The study of the soil microbiome allows finding out the complex relationships with the biotic and abiotic environment, highlighting the changes in the microbiome as well as the role of the microbial communities.

The extracellular content biosynthesized by microorganisms is also involved in the qualitative and quantitative processes or evolution of soil organic matter, in microbial mobility, increasing heterogeneity and stability of aggregates. The importance of these microbial secondary metabolites is also reflected in the liberating role of nutrients through the degradation of organic matter, in the identification of priority microbial activities, but also as a sensitive indicator of ecological changes. Their monitoring can be ensured by evaluating the biotic component of the soil. The impact on the possible edaphic ecosystem requires a deeper understanding of the relationships between the strategies used and the synthetic microbiome introduced into the soil (Lehmann et al., 2020; Fierer et al., 2021).

An increase in carbon sequestration rates, in cultivated soils, also intervenes in reducing atmospheric carbon concentrations, and attempts to promote soil carbon sequestration can provide direct benefits for its health and productivity, taking into account the importance of organic carbon concentrations for the sustainability of agriculture (Amelung et al., 2020; Bossio et al., 2020).

By manipulating microbiomes, it is possible to enhance the carbon sequestration capacity of soils. Consequently, new strategies appear necessary to accelerate the rates of soil carbon sequestration, especially in cultivated soils, where carbon stocks have been reduced by agricultural activities and where practically their increase has been achieved exclusively through direct intervention. Through new strategies to accelerate carbon sequestration in cultivated soils, both the mitigation of climate change and the improvement of food security are contributed.

The research aimed to identify the traits, respectively the microbial properties that can lead to changes in soil carbon dynamics, to the possibility of accelerating carbon sequestration, through the influence exerted by the composition of synthetic microbiomes and by capitalizing on their specific characteristics.

MATERIALS AND METHODS

Soil characteristics of Mollic Histic Gleysol (Salinic) (WRB) were: humus content 29.8%, organic C 17.3%, organic matter 51%, pH value 5.54, total nitrogen 1.740 mg·kg^{-1}, N-NO_3 2106 mg·kg^{-1}, P_{AL} 45 mg·kg^{-1}, carbonates 0.4%, microelements (Fe 23.259 mg·kg-1, Mn 144mg·kg^{-1}, Cu 55.3 mg·kg^{-1}, Zn 67.9 mg·kg^{-1}), clay content <0.002 of 38.6%.

In the experiment, carried out in control conditions at NRDISSAE, Bucharest, in summer 2023, the influence of the microbiomes composition M1-M9 on the soil biodiversity and dynamics of soil C was analysed after a period of 60 days from the application of 2 ml of inoculum/pot with concentrations between 3.26-5.42x10^3 ufc/ml for fungi and between 6.35-8.74x10^6 viable cell/ml for bacteria. The bioassay pots contained Mollic Histic Gleysol (Salinic) were inoculated and incubated at 27^{0}C, at a constant humidity of 60% of the soil field capacity and maintained for a period of 60 days, under the same controlled conditions. At the end of this period, soil samples were collected for analysis. Five replicates were used for each experimental variant.

The criteria for microbiomes selection and creation of synthetic microbiomes M1-M9 sought to ensure biodiversity and adaptability, microbial biomass production, increased exometabolic production and involvement in the modelling of organic fractions, the presence and rhizospheric activity, the predominance of fungal microflora, the presence of fungi endosymbionts and antagonists. Within each bio-system, the principle of using and grouping microbial isolates was respected in accordance with the biomass/metabolites/CO_2 production relationship. Thus, bio-systems M1-M9 contain combined microorganisms, between 7-12 isolates/microbiome.

Microbial communities belonging to microbiomes M1-M9 contain bacterial, fungal, diazotrophic and mycorrhizal microflora. Before inoculation, the microbial species are kept in pure cultures, on culture media (Topping, Czapek, LB, BHI, MRS, King, YEM, MMN) at a temperature of 4°C. The taxonomic study used the morphological criteria by optical microscope (MC 5.A) examination and measurements according to determinative manuals: Bergey & Holt (1994) for bacteria and Domsch & Gams (1970) for fungi, to assess microbial diversity.

Microbiomes M1-M9 contain microorganisms belonging to bacterial genera: *Pseudomonas, Bacillus, Azotobacter*, *Rhizobium, Acetobacter, Paenibacillus, Serratia, Thiobacillus, Streptomyces,* to the fungal genera *Chaetomium, Arthrobotrys, Cunninghamella, Myrothecium, Trichoderma, Torula, Rhizopus, Aspergillus, Penicillium, Stachybotrys, Mortierella, Cladosporium, Humicola, Micromonospora, Acaulospora, Glomus.*

The diversity indices used measured the evolution of the number and distribution, evenness of species in communities inoculated with microbiomes (abundance, evenness, Shannon-Wiener and Simpson indices).

The quantitative analysis of the microflora from the soil variants inoculated with the M1-M9 microbiomes were performed according to the method of soil serial dilutions. Culture media specific to microflora were used, respectively: Nutrient Agar (NA) for heterotrophic aerobic bacteria (Difco) and (PDA) for saprophytic fungi (Merck). Colonies developed after 4-7 days of incubation (27°C) were counted and the results expressed as total number of bacteria (TNB)-viable cells $x10^6$ and total number of fungi (TNF)-colony-forming units (cfu) $x10^3$ reported per 1 gram of dry soil (Dumitru & Manea, 2011) .

Soil microbial biomass was determined according to the fumigation-extraction method. Soil samples were fumigated with $CHCl_3$ at 20°C for 24 hours, extracted with K_2SO_4 and filtered using cellulose ester filter. Soil microbial biomass carbon was calculated according to the Standard-SR-ER-ISO-14240-2-(2012), as average of five replicates/variant.

For separating water-soluble subfraction D, fulvic fraction of Mollic Histic Gleysol (Salinic) was extracted and fractionated by adsorption on activated charcoal, to following by serial elution with acetone, NaOH and finally in distilled water, followed by its migration on a ascendant chromatograms (Votolin et al., 2022).

Fluorescence of dissolved organic carbon in the fulvic sub-fractions of microbiome-inoculated soil was extracted, treated with fluorochromes, and its distribution revealed by specific ascending chromatography (Wang et al., 2021). The photographic images obtained under 350 nm UV illumination revealed the qualitative differences between the final phases of microbiome application, as well as aspects related to the different densities of the newly synthesized biochemical composition, the distribution of the material and its complexity, highlighted by the affinity for fluorescence.

RESULTS AND DISCUSSIONS

Microbial communities have been selected to constitute synthetic microbiome variants (M1-M9) that were inoculated into Mollic Histic Gleysol (Salinic). Due to their microbial carbon generating character, diversity indicators were used to assess the effects on biodiversity and soil carbon dynamics. Taxonomic composition and relative abundance in bacterial and fungal communities control soil and variants inoculated with synthetic microbiomes are presented in Figure 1 and Figure 2.

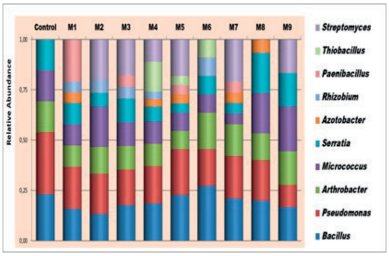

Figure 1. Taxonomic composition and abundance of bacteria in soil inoculated with microbiomes M1-M9

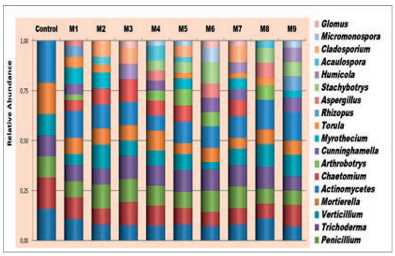

Figure 2. Taxonomic composition and abundance of fungi in soil inoculated with microbiomes M1-M9

From an ecological point of view, increased number of species favours community functionality and stability in the face of perturbing factors through complementarity mechanisms, such that after inoculation with M4 and M5 microbiomes, soil microflora had different abundance of component species, the more numerous individuals representing new inoculated species integrated in the communities of soil. They use more of the available resources, increasing the biosynthesis of exometabolites. These species (e.g. as those from genera *Bacillus, Pseudomonas, Streptomyces, Trichoderma, Penicillium, Chaetomium, Mucor,* in variants M4-M5) developed abundantly in soil compared with other inoculated or endemic species. Also, through selection mechanisms, individuals from different species originating from the M1-M9 microbiomes contribute differently to the overall functionality of the soil microbial community after inoculation, because they differ from each other in biosynthetic efficiency. As a result, the increased abundance in microbiome-inoculated soil likely stems from the richer content in adapted and efficient individuals belonging to certain species in the microbiome community.

Homogeneity (evenness) is an important factor in assessing maintaining functional stability and increasing productivity. In the analysed communities, it refers to the distribution of individuals by species (Table 1).

Table 1. Diversity indices and evenness of microbial communities in control and soils inoculated with synthetic microbiomes M1-M9

Microbiome Variants	Diversity indices		
	Shannon-Wiener index (H')	Evenness (ε)	Simpson index (D)
Control	2.447	0.916	0.910
M1	2.993	0.908	0.946
M2	2.883	0.903	0.941
M3	2.890	0.910	0.942
M4	3.054	0.922	0.950
M5	3.047	0.897	0.947
M6	2.921	0.894	0.943
M7	2.979	0.903	0.945
M8	2.825	0.907	0.938
M9	2.886	0.914	0.941

Thus, the homogeneity is high (ε=0.922) in the soil community inoculated with the M4 microbiome where almost all species have a similar distribution and the uniformity is lower (ε=0.894) in the soil community inoculated with the M6 microbiome, reflecting a more heterogeneous distribution by species. By intensifying the expression of the functional traits of the species, in the case of soil inoculated with M1-M9 microbiomes, compared to the control communities, they may be more resistant to stress and various disturbances, less susceptible than in the case of non-inoculated soil.

As a measure of diversity, the Shannon index used can also be constituted as a measure of entropy, both of which have conceptual similarities, and therefore entropic and diversity indices share many of the characterization axioms, but do not mean that they are equivalent. Thus, the entropy indices are linear functions of abundance/probability, while the diversity indices used are non-linear and the average diversity of the number of microbial communities is not the average of their diversities. The adoption of this index assumed the consideration of appropriate conceptual differences, based on the evaluation of the real abundance of the different species present in the inoculated soil. In general, indices condense into one number relevant information on the diversity of an edaphic system, using complex data about that system (Davydov et al., 2016; Tucker et al., 2016; Kang et al., 2016; Buckland et al., 2017; Grabchak et al., 2017; Butturi-Gomes et al, 2017; Botta-Dukat, 2018). The use of this index depended on the way in which it is mathematically constructed, on the method of ecological interpretation, as well as on the context of the study, which assumed the observation of diversity changes induced by inoculation, the estimation of their evolution according to the level of biodiversity, the understanding of the interactions between biodiversity and trophic level reached, evaluation of the effect of change in diversity.

In this study we focused on species diversity in soil inoculated with different microbiomes because species diversity is considered the most common form of soil diversity analysis.

The values determined for the Shannon index in the bio-systems analysed varied between 2.447 for non-inoculated Mollic Histic Geysol (Salinic) and 3.054, respectively 3.047, for the same type of soil inoculated with microbiomes M4 and M5, respectively. In general, the inoculated microbiomes generated Shannon

index values above 2 and can be considered to have average diversity. Shannon diversity index values above 3, as in the case of the influence of the application of M4 and M5 microbiomes, are considered values of a high diversity.
In the case of applying the Simpson diversity index (D) in the analysis of diversity in Mollic Histic Geysol (Salinic) inoculated with microbiomes M1-M9, the highest value registered in the case of the soil inoculated with the M4 microbiome (D=0.950), which indicates an increased number so species and uniformity, respectively a good species diversity of the community. The lower values determined in the case of soil inoculated with microbiomes M2, M9 (D=0.941) and M8 (D=0.938), indicated a lower diversity of these heterogeneous communities. Compared to the Shannon-Wiener index, the Simpson index is an indicator of α-diversity and the rare species in the inoculated soil communities have a smaller role in the calculation of the index, in contrast to the common species that acquire a more important role. In general, the Shannon Index emphasizes the abundance component, sparse cover types, and the Simpson Index preferentially uses the evenness component and dominant cover types.
The value of the presence of endemic species can be diluted by the evaluations of such indices. In general, endemic species have characteristic soil type distribution and specificity, and for indices, these endemic species become only a few more species in the microhabitat. Consequently, analysis of the endemic status was considered, in the case of artificial introduction of M1-M9 microbiomes. The results revealed that none of the introduced microbiomes significantly influenced the endemic soil microbial presence and dynamics.
The monitoring of synthetic microbiomes can be achieved not only based on changes in some abiotic variables (soil nutrients, pollutants, soil structure), but especially through biological indicators (where microbial strategies are used), represented by quantitative and qualitative assessments of microflora, biomass, carbon dissolved organic matter. The assessment of biodiversity in the soil was carried out by taxonomic tests based on the analysis and classification of different parts of the microbial material. The priority, in the case of studying the biodiversity of the soil inoculated with the M1-M9 microbiomes, included the determination of the newly formed microhabitats, the possible factors that intervene in vulnerability/favouring the increase of diversity, following the applied treatments, by changing the supply of energy and nutrients, the creation of microhabitats and the new biodiversity in the soil.
The microbial bio-systems active *in situ* were expressed by the presence and level of their activity in the soil, by the preponderant involvement of the fungal microflora in the circuits of major elements (carbon, nitrogen, phosphorus).
Biodiversity is important for the viability of edaphic ecosystems and its loss leads to functional degradation and a rapid evolution towards total collapse. Through human activities, this characteristic has worsened and reached critical values, due to high extinction rates of edaphic species and the generation of changes in the structure and functionality of the affected ecosystems. The molecular and ecological bases of functions at the microbial community level are reduced, as well as the stability, robustness of their functions, extent, diversity, structure, size, as community properties. The influence of these factors on synthetic community composition may vary in time and by ecosystem. The diversity is a challenge in the direction of relationships between species composition, function and community dynamics (Bunge et al., 2013).
Soil microbial communities are essential in maintaining soil quality. They are an important part of the ecosystem (Jessup et al., 2004; Dıaz de Otalora et al., 2021), and underground generators of soil benefits, too (Matei et al., 2019). There are differences between the compositions of the microbial communities in the experimental sites compared to the conventional systems, between the increases of microbial biomass or between the levels of their activities that appear much intensified (Frac et al., 2018; Fenster et al., 2021; Lujan Soto et al., 2021). Also, the aboveground activities of these soils can have an influence on the components of artificial microbiomes, as well as on the bacterial and fungal microflora of the underground ecosystem (Hermans et al., 2017; Banerjee et al., 2019).
Different studies have shown that synthetic microbiomes improve soil fertility through the

capabilities of intervening in biogeochemical cycles, C storage and providing greater plant diversity through soil microbial processes.

Microbial activity in the rhizosphere has an impact on soil hydrophobicity, offering the possibility of increasing efficiency by indirectly manipulating soil microbiomes, changing management practices or directly by modifying endemic microbial communities, respectively by introducing appropriate microorganisms with a functional regenerative role (Matei et al., 2016a; Hu et al., 2018; Hartman et al., 2018).

The sequestration of soil carbon involves a faster accumulation than its loss over time. Thus, multiple approaches (e.g., through rooting depth, through cover crops) have attempted to alter this balance in agricultural systems, but one can intervene in increasing soil carbon sequestration capacity and through a direct manipulation of microbiome composition soils by introducing synthetic microbiomes. Through this approach or in combination with other types of approaches, the potential of microbial activities that control the net flux of carbon within soil systems can be harnessed.

The soil microbiota controls the processing rates of organic carbon inputs as well as biochemical stabilization through reactions with mineral surfaces. It can also intervene in the transformation of organic carbon into soluble or gaseous forms of carbon that can leave the system, form stable micro-aggregates with a diameter below 250 μm containing clays, sesquioxides and microorganisms (Totsche et al., 2018). Microbes ensure the protection of soil carbon reserves from mineralization processes or the reduction of losses in the form of organic carbon particles, through erosion (Hartmann et al., 2022).

In general, the soil microbiome is particularly complex and its multiple specific contributions to carbon dynamics are little appreciated (Fierer et al., 2017). The processes of stabilization over time and carbon retention in soil are complex and highly variable in time and space, with relatively little estimated results, due to biotic and abiotic interactions (Lehmann et al., 2015).

Perhaps research in the field will also focus on evaluations of the functional contributions of synthetic microbiomes in soil, not just on compositional changes. Microbial communities can contain functional variations, given similar compositions, or, on the contrary, compositionally different microbial communities can have similar functional potential due to functional redundancy (Castaneda et al., 2017; Louca et al., 2018).

Strategies aimed at sequestering carbon cause changes in the soil environment so that different physicochemical properties of soils support microbial communities with different functional profiles. Microbial functions for soil and understanding how these functions change is also essential in estimating the overall impact on the soil ecosystem (Bahram et al., 2018)

The functions change with depth, so that the functions for carbon sequestration, for the metabolism of nutrients, nitrogen and phosphorus are lower than in surface soils. Investigating changes in active microbial communities deep in the soil profile after the introduction of synthetic microbiomes can improve the impact of regenerative practices (based on them) on soil health (Rchiad et al., 2022).

Analysis of biodiversity in soil inoculated with synthetic microbiomes provide some examples of how biodiversity indicators convey information about what is happening with synthetic microbiomes in a soil, as well as support for the development of ways to control the activities and processes involved in ensuring his health. The quantitative assessment of the edaphic heterotrophic bacterial and saprophytic fungal microflora in Mollic Histic Gleysol (Salinic) was carried out after 60 days from inoculation with M1-M9 microbiomes. In all experimental variants, after the introduction of selected synthetic microbiomes over the resident microflora, a stimulation of their numerical growth was found. Thus, the bacterial microflora showed growth rates of up to 54%, at 60 days after inoculation, in the case of using the M4 microbiome (344.32 x 10^6 viable cells x g^{-1} dry soil). In the non-inoculated variant, the increase within the same time period was 3.12% (19.9 x 10^6 viable cells x g^{-1} dry soil). The introduction of the M1 microbiome into the soil caused quantitative increases in the fungal microflora, in the final of period compared to the initial values (respectively from 45.36 x 10^3 cfu x g^{-1} dry soil to 86.34 x 10^3 cfu x g^{-1} dry soil, in the case of the M1 microbiome). Also, the quantitative increases of the fungal microflora

60 days post inoculation were significant compared to the initial values, after the introduction of microbiomes M4, M5 and M7 and non-significant, in the case of microbiomes M6, M8 and M9 (Figures 3 and 4).

Figure 3. Soil bacterial microflora after 60 days from inoculation with microbiome M4

Figure 4. Soil fungal microflora after 60 days from inoculation with microbiome M4

The microbiomes M1 and M5 containing within the community bacterial microflora with antagonistic characteristics (*Bacillus* sp.,) and hyperparasitic fungi (*Trichoderma* sp.,) influenced the quantitative increases of the microflora. Also, the microbiomes M4 and M7 containing free-living microorganisms and symbionts with an important role in the nitrogen cycle (*Azotobacter* sp., *Rhizobium* sp.) and fungal endosymbionts with a role in the phosphorus cycle (*Glomus* sp.), influence the growth with up to 30% of the soil microflora, after 60 days from inoculation. The influence of the presence of the free diazotroph *Azotobacter* on the quantitative growth of the microflora could be due to the biosynthesis of a series of biologically active substances, to its biodegradative capacity on aromatic compounds, as well as to the role played in ensuring the mobility of metals in the soil. Also, the species of *Rhizobium* present in the microbiome community, as an endosymbiont, after inoculation in the soil obtains nutrients from the plant, produces nitrogen in the inoculated soil, through biological fixation, and mycorrhizal fungi (*Glomus* sp.) improve nutrient absorption and stimulate biodiversity.

Microbial biomass of soil inoculated with M1-M9 microbiomes correlated well with the level of microbiome activity and diversity. Thus, the biomass level directly reflected the intensity of the activities of the microbiomes in Mollic Histic Gleysol (Salinic), and the identical culture conditions allowed the evaluation of the influence of the inoculated microbiomes on the evolution of the biomass (Figure 5).

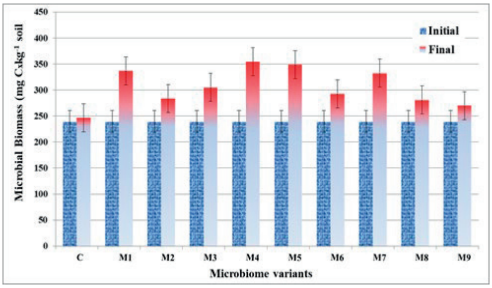

Figure 5. Microbial biomass of the soil inoculated with microbiomes M1-M9

Under experimental conditions, biomass accumulations showed variations between 12-48% for the 9 variants of inoculated with microbiomes. The biomass produced by microbiomes M1, M4, M7, after introduction into the soil, reached values of up to 354 mg C x kg^{-1} soil (M4) after 60 days from inoculation and represented the experimental variants with up to 48% increase in the amount of microbial biomass. In the variants inoculated with microbiomes M2 and M6, the microbial biomass content increased by 18.56% (M2) to 22.14% (M6), reaching a biomass of 272 mg C x kg^{-1} soil. The amount of biomass accumulated had the lowest level in the case of Mollic Histic Gleysol (Salinic) inoculated with the M9 microbiome, where the biomass increase was of only 12.81% compared to the initial determinations. Biomass accumulations partially reflect the degree of integration into the edaphic community of introduced bacterial and fungal microflora, the functional and ecological compatibility with endemic microflora. Increasing the microbial biomass in soils by inoculating synthetic microbiomes can be an opportunity to create complex reservoirs of microbial life on the basis of which the effect of microbial monocultures of some performing microbial strains, the vulnerability of the edaphic bio-system to pathogens and extreme conditions can be avoided (Wagg et al., 2019; Averill et al., 2022; Mishra et al., 2022). Increasing and exploiting the dimensions of microbiomes in soils generates the necessary potential for the transformation/control of these bio-systems, as well as for microbiome monitoring and conservation. Attempts to increase biodiversity, microbial biomass, especially in managed soils, aim to restore or improve the composition of microbiomes with functionally and productively diverse bacterial and fungal microflora. Microbial biomass ensures the immobilization, mineralization of nutrients and the formation of soil aggregates, and during its quantitative development, the increase in carbon content (C) can provide between 1-3% of soil organic carbon (SOC) (Seita et al., 2012; Wu et al., 2020). Thus, the carbon in the microbial biomass can be considered as part of the active, unstable fraction, concentrated especially in the surface area of the soil and participating in the formation of SOC, alongside the passive fraction with long-term stability (Alvarez et al., 2016). Variable relationships are also thought to exist between microbial biomass carbon and soil physical and biogeochemical properties. The synthetic microbial bio-systems from experiment were active and caused increases in microbial biomass in the inoculated variants, the species in the microbiomes being grouped in accordance with the biomass-metabolites-CO_2 relationships.

The dissolved organic carbon compounds from the soil inoculated with the M1-M9 microbiomes were analysed because they represent the organic matter extractable in water, and their fluorescence characteristics were used because they allow the highlighting of active but also labile reserves of biosynthesized organic matter (Matei et al., 2016b; 2023). For example, free-living diazotrophs and other species of soil microbiota produce water-soluble compounds that can be of various colours, dark brown, yellow-green to purple, green with yellow-green fluorescence, and blue with blue-white fluorescence. The process of biosynthesis of such fluorescent compounds is carried out at high metabolic rates and they are involved in the protection of enzyme systems.

The process of microbial carbon biosynthesis in soil, under certain standard conditions and for short periods of time, had an effect on SOC through labile, water-extractable and fluorescent substances biosynthesized by endemic and inoculated microflora.

In addition, the fluorescence of protein, organo-mineral and humic substances, present in the organo-mineral reserve of the soil, is added. By visualizing their presence and monitoring their distribution, some of the short-term effects of various microorganisms with a biosynthesizing role, from the analysed soil, on the composition of the organic matter extractable in water and the possible options for intensifying C storage were elucidated.

Extracts from the water-soluble sub-fraction of fulvic acids were used in order to highlight the most significant fluorescent components of organic matter (OM) extractable in water, components that can accumulate and integrate into organic matter, as a result of microbial biosynthesis carried out in short term.

The fluorescent components integrated into the structure of water-extractable organic matter from the soil inoculated with synthetic microbiomes were analysed and each of the sub-fractions related to the soil inoculated with microbiomes M1-M9 highlighted the presence and level of association of biosynthesized microbial organic compounds.

The fluorescent components highlighted (by imagistic technique) the relative degree of formation and storage of newly biosynthesized carbon in the structure of the organic matter, compared to the composition of the initial organic component, in a short period of time (60 days). The distribution of this biosynthesized carbon was highlighted in the specific case of each type of microbiome applied to the soil (Figure 6).

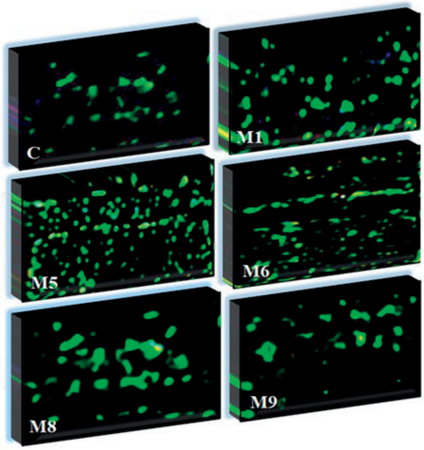

Figure 6. Fluorescent components of Mollic Histic Gleysol (Salinic) after 60 days from inoculation with M1-M9 microbiomes

The sensitivity of the fluorescent components biosynthesized by the microbiomes M1-M9, but also their complexity, was compared with the initial spectrum of the endogenous organic component of the non-inoculated soil, which allowed highlighting the storage possibilities of biosynthesized microbial C, in Mollic Histic Gleysol (Salinic), after inoculation. Through the dynamics of the processes and the level of microbial activities and also by the composition of the extracellular organic component introduced into the soil, it is possible to highlight the direct impact on the organic component, to estimate the efficiency of the mineral protection of the deposited carbon, as well as on the biotic processes of synthesis/decomposition in soils, which appear as relatively complex, correlated and interdependent phenomena. After the inoculation of the M1-M9 microbiomes, the dynamic changes produced in the architecture, composition, concentration of biosynthesized C and of the dissolved organic matter (DOM) were highlighted by means of fluorescence characteristics (Wang et al., 2016; Fox et al., 2017; Arai et al., 2018).

Thus, the fluorescence variations of the C compounds soluble in water would also be due to a possible increase in the lability of the analysed soil carbon, to the fixation in the organic complexes of the biosynthesized compounds simultaneously with the improvement of the activities and accumulations of microbial biomass. Biosynthesized organic components (proteins, amino acids, fulvic acid-like substances a.s.o.) detectable by fluorescence revealed an abundance of the composition consisting mainly of fulvic materials with different molecular weights, aromaticity and condensation, with the increase in the number of species in the microbiome. Also, if the biosynthesized compounds would influence soil acidity, the dispersion of fluorescent colloidal associations and aggregates, desorption of organic materials, the release of C compounds and the reduction of the fluorescence level could occur. Basically, the biosynthesized microbial C supplements the dissolved organic carbon in the soil and can also stabilize on mineral surfaces. Through electrostatic interactions, based on the compression of the charged layer around an ion, they manifest themselves until the repulsive forces are overcome by the attractive forces. Also, if the concentration of the soil solution ends up causing competition for the sorption sites, they can desorb, causing variations in the fluorescence level, due to the release/adsorption of the constituents.

In general, water soluble biosynthesized C compounds, constitute the most active and also the most mobile fraction of soil organic matter. Also, any increase in organic matter content influences soil health parameters (e.g. available water capacity).

In addition, soluble organic C compounds also play a critical role in many soil processes due to their mobility and reactivity at the soil-water interface.

Monitoring the variations and origin of dissolved organic matter is important in soils because they are factors of biotic functioning in various bio-systems, in the cycle of nutrients and their transport, the solubilisation of organic pollutants, the mitigation of ultraviolet radiation and the control of the bioavailability of metals.

Highlighted variations in dissolved organic carbon were due to characteristics of microorganisms in the composition of microbiomes that functioned metabolically differently and directly influenced soil processes.

Through the specific content of extracellular metabolites released, the microbiomes (M1-M9) inoculated into the soil influenced the dynamics of the energy potential.

Synthetic microbiomes also produce exometabolites that increase organic C retention in various soils, but can also cause a decrease in organic C retention in other soils. These additional inputs of labile organic carbon from microbial secondary metabolism activate C stocks and accelerate microbial decomposition of SOM (Lehmann et al., 2015).

For particularly complex soil conditions, ways can be created in which synthetic microbiomes can be used to promote soil carbon sequestration.

Microbial bio-systems M1-M9 from experiment were realized and selected according to the qualitative/quantitative composition and characteristics of exometabolic compounds.

Microorganisms in synthetic microbiomes are active players in the C cycle in the soil, and these microbial carbon pumps are to practically realize the microbial production of a set of organic compounds as well as their subsequent stabilization.

The composition and use of microbiomes appears important for the evolution of soil C. There are links between the composition of a microbiome and rates of synthesis /decomposition of other compounds, as well as increases in soil C storage.

The extracellular microbial content constitutes a competitive advantage through which microbiomes can influence nutrient transport, energy potential dynamics and tolerance to stress in the soil.

Also, through their intervention you can protect/prolong: the activity of soil processes; stimulating the qualitative and quantitative evolution of organic C; facilitating microbial movements; ensuring the stability of soil aggregates. Also, microbiome composition influences soil processes only if the microorganisms differ in how they function metabolically.

CONCLUSIONS

Soil inoculation with synthetic microbiomes M1-M9, selected for the biosynthesis characteristics of organic compounds, allowed obtaining experimental data regarding their influence on the evolution of soil biodiversity;

The soil inoculation with synthetic microbiomes M1-M9 influenced the rate of quantitative growth of microflora, biomass accumulation and biosynthesis of soluble organic C compounds from the fulvic subfraction, according to the level of their exometabolic activities.

Bacterial and fungal microflora showed significant growth rates compared to the initial values of uninoculated Mollic Histic Gleysol (Salinic), and in the case of inoculation of M4 and M5 microbiomes reaching increases of up to 54%, 60 days after inoculation.

The microbiomes M1 and M5 including bacteria with antagonistic characteristics, hyperparasitic fungi contributed to increases of up to 32% of the total soil microflora.

The microbiomes M4 and M7, which contain diazotrophs and mycorrhizal fungi, stimulated increases of up to 30% of total of counts of soil microflora.

The microbial biomass produced after the inoculation of soil with artificial microbiomes showed an increase of up to 48%, reaching values of up to 354 mg C x kg^{-1} soil (M4), at the end of the experiment.

Microflora from each microbiome influenced differently the distribution of the fluorescent dissolved organic carbon from each water soluble fulvic subfraction of Mollic Histic Gleysol (Salinic).

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PLANT MILK - ALTERNATIVE FOR DAIRY PRODUCTS. RHEOLOGICAL CHARACTERISTICS AND NUTRITIONAL COMPOSITION

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Abstract

The objective of this work was to evaluate the physical, chemical and rheological characteristics of vegetable milk of soybean and almond (homemade prepared, and some types of plant milk purchased from Romanian supermarkets). Vegetable milk is a colloidal solution obtained in the form of water extract from swollen and ground soybeans or other grains (rice, oats, almonds a.o.). The vegetable milk samples (soymilk and almond milk) were prepared from analyzed grains and then a set of chemical and physical characteristics of the milk were assessed. The present study paper investigated the moisture and total dry content substance (TDC), total mineral content - ash content, macronutrients content (fat, protein and carbohydrate content) for soybeans, almonds and vegetable milk samples.

Key words: *soybeans, almonds, plant milk, rheological characterization, moisture, fat, protein content.*

INTRODUCTION

Milk is the lacteal secretion obtained by the complete milking of one or more healthy milch animals", thus technically differentiating animal milk from plant milk (Fructuoso et al., 2021). Globally, cow's milk and its derivatives are consumed by over 6 billion people (Fructuoso et al., 2021), mainly due to their protein and calcium content (Fructuoso et al., 2021; Silva et al., 2019; Vanga & Raghavan, 2018).

Despite the dietary benefits offered by milk, some people present with disorders such as cow's milk allergy (CMA) and lactose intolerance (65-75% of individuals) (Fructuoso et al. 2021; Munekata et al., 2020; Vanga and Raghavan, 2018; Mäkinen et al., 2016).

Taking into account all the disorders produced by animal milk, the main alternative would be to replace it with vegetable/plant) milk. Plant milks are liquid emulsions obtained by the decomposition of plant tissues extracted in water. Plant milk is a stable emulsion of oil, water and protein (Micula et al., 2016; Bonifacio da Silva et al., 2009; Li et al., 2016). The stability of the final product and the size of the emulsion particles depend on the nature, biological properties of the raw material, decomposition and storage conditions of the raw material. To be functional, plant-based milk alternatives must be able to meet the needs of those who choose different lifestyles, to increase energy, to fight aging, fatigue, stress, promote cardiovascular health and reduce some mineral deficiencies (e.g.: iron and zinc) (Milovanovic et al., 2023; Omoni and Aluko, 2005; Kundu et al., 2018).

Plant-based drinks show variable composition on the amount of macro- and micronutrients and the presence of bioactive compounds and anti-nutritional factors (Munekata et al., 2020). These differences are due to the type of plants used for the preparation of vegetable milk. For the manufacturing process of vegetable milk, are used either legumes (e.g.: soy, chickpea), nuts/nut kernels (e.g.: almonds, cashew nuts, hazelnuts and Brazil nuts, seeds (sunflower, sesame), cereals (rice, oats) or pseudocereals (e.g. quinoa).

Increasing demand for non-dairy alternatives has been driven by concerns about dairy milk consumption such as socio-religious beliefs against the consumption of animal products, allergenicity associated with its constituents, phobia for contracting diseases and the philosophical and ethical practice of veganism (Panghal et al., 2018), The most popular plant-based milk beverages are obtained from almonds, oats, soy, cashew and coconut, or a mixture of those (Vanga and Raghavan, 2018)

An alternative to dairy products is soymilk and it has long been a traditional drink in Asia - China, Japan and other parts of this continent (Micula et al., 2016; Liu, 1997).

Soybean (*Glycine max*) is an important source of macronutrients, minerals, vitamins and many bioactive compounds (mainly isoflavones) – which are important for the health benefits offered. Bioactive compounds increase the protection against cardiovascular disease, cancer, osteoporosis, dermatologic diseases and neurodegenerative disorders (Aydar et al., 2020). Soybeans are also a source of essential monounsaturated and polyunsaturated fatty acids, including linoleic and linolenic acids, and are cholesterol-free (Karimidastjerd and Gulsunoglu Konuskan, 2021; Silva et al., 2020).

Soy milk has a lot of nutritional compounds, high digestibility and low production cost and does not contain lactose, cholesterol (Sethi et al., 2016). Because soy milk contains various proteins that can cause allergic reactions, people with cow's milk allergy may also have reactions against soy (Karimidastjerd and Gulsunoglu Konuskan, 2021; Silva et al., 2020). Due to some antinutrients, such as phytate, oxalate and saponins that form insoluble compounds as a result of the reaction with mineral cations, soy milk has a negative effect on health by decreasing the bioavailability of vitamins and minerals (Aydar et al., 2020).

The most consumed milk alternative among plant milks is almond milk. Almonds (Prunus dulcis) have become an important food for a modern, healthy lifestyle because they contain many beneficial components (Sathe et al., 2002). Almonds contain a large amount of soluble sugars, proteins, lipids, minerals, fibers and antioxidants (Silva et al., 2020).

Almond milk has a multitude of benefits such as controlling blood lipids, preventing anemia, lowering the risk of heart disease, and the antioxidant effect by protecting against free radicals. Almonds can also have a prebiotic action due to the content of arabinose that helps reduce serum cholesterol levels. Unfortunately, almonds have allergy potential in individuals (Sethi et al., 2016; Silva et al., 2020). Almond milk is rich in vitamin E (6.33%) in the form of α-tocopherol and manganese. Vitamin E is a powerful antioxidant with a protective role against free radical reactions (Karimidastjerd and Gulsunoglu Konuskan, 2021; Chalupa-Krebzdak et al., 2018).

The purpose of this study was to develop and evaluate two varieties of plant-based milk (soybean and almond). The evaluation consists in determining the rheological, physical, chemical, nutritional and sensorial proprieties. The plant-based milk, taken into the study, was both prepared by us and purchased from supermarkets.

In the present study were evaluated the physicochemical characteristics of plant milk beverages: vitamin C content, pH, acidity, salinity, total content of soluble solids - TSS, sensorial and rheological characteristics; and we investigated total mineral content (ash), moisture (water content) and total dry substances content - TDC, macronutrients content (fat, carbohydrates and protein content) on soybean and almond kernels used for homemade plat milk and for plant milk samples.

MATERIALS AND METHODS

Reagents and chemicals

All reagents employed in this study were of analytical grade and were purchased from Merck (Germany), Sigma-Aldrich (Germany) and Fluka.

Materials and preparation of samples

In this study, we prepared plant milk samples at room temperature from almonds and soybeans. Fresh spring water was used for preparing the plant milk. Soymilk was also prepared by boiling. The preparation method at room temperature (220C) (without heating or boiling) is also called the raw vegan preparation method. The products obtained through this process are called raw vegan products.

In the present study, soybeans and almond kernels purchased from Romanian supermarkets and plant-based milk (which was prepared at home or purchased from the supermarket) were evaluated. The plant-based milk samples purchased from local supermarkets are described in Table 1. In the selection of products, brands with significant domestic sales but with different ingredients were taken into account.

Table 1. Ingredient lists of the commercial plant beverages studied

Products	Ingredients lists
A Almond milk	water, almonds (2.3 %), calcium (calcium carbonat), sea salt, stabilizer agent (agar gum, gellan gum), emulsifier (lecithin), natural flavour; 100% Mediterranean almonds, no sugar, no sweeteners, low in fat, source of Ca and vitamins: B2, B12, D2, and E
B Soymilk	water, decorticated soy beans (8%), sugar, acidity corrector (potassium phosphates), calcium (calcium carbonate), stabilizer agent (gellan gum), source of Ca and vitamins:B12, D2,free for dairy and gluten

Preparation of homemade vegetable milk was achieved using a vegetable milk Biovita-10 device, which contains an incorporated blender. This device can grind and boil, not needing another device to grind, shred and blend.
For the preparation of raw plant milk, the ratio between the plant quantity and the spring water quantity was 1: 3 for almond milk and 1: 5 for soymilk. It was used bottled spring water bought from the supermarket, from local distributors. Distilled water was not used because it was desired to improve the mineral intake of the vegetable milk obtained.
Before introducing them in the device, the soybeans/or almond kernels had been soaked in water for twenty hours. Subsequently, the soaking liquid was drained and then the plants (the soybeans or almonds) and water were introduced into the device, during the process, the soybeans or almond kernels were ground very finely.
Finally, the resulting mixture (of soy/or almond and water), was filtrated through a very fine sieve (the diameter of the sieve being smaller than 0.5 mm), thus obtaining both the vegetable milk and the mass of solid vegetable substance (MSS) separated through draining. The experimental analysis has been conducted on both the raw material employed for the milk production and on the resulting products (the remaining solid mass (MSS) and the plant milk).

Four plant-based milk samples marked A, B, AM and SM were evaluated and analysed. A (almond milk) and B (soymilk) are two kinds of plant milk purchased from supermarkets. The analysed samples from homemade vegetable milk were AM, SM and BSM. The BSM sample is a soymilk sample obtained by boiling. The AM (almond milk) and SM (soymilk) are raw plant milk samples obtained by us.
Based on the analysed samples, the assessment of the following aspects has been made: the dry matter, content humidity, ashes, vitamin C content, pH, the Brix degree, salinity, and rheological behaviour.

Physical and chemical analyses

Weight determination. Soybean grains and almond kernels weight has been measured by using a digital balance with a sensitivity of 0.001 g.
The determination of water content and total dry weight. The moisture and total dry weight of samples were was made by using a Memmert drying oven, by drying at $105 \pm 2°C$ until they reached constant weight (in 4 hours). The water content actually represents the weight loss achieved by heating at the temperature indicated by the analysis method until reaching the constant mass.
The ash content (total mineral content) was evaluated by incineration at $500 \pm 15°C$.
The term “ashes” designates the residue obtained from the alimentary products after the total elimination of the organic substances, by calcination and evaporation of water.
The determination of the total soluble solids TSS (the Brix degree) and of salinity in the milk samples has been achieved by employing the KRÜSS (Germany) optometric equipment.
The determination of pH has been obtained with the help of a pH-meter (Tester pH ExStick™ PH-100, Extech Instruments a FLIR Company) calibrated with pH 4 and 7 buffers.
The vitamin C content determination for the plant milk samples
The chemical methods of dosage for the ascorbic acid are based on its reducing property. The ascorbic acid is transformed by oxidation in dehydroascorbic acid.
The redox indicator 2,6-dichloroindophenol is a weak oxidizing agent with a mild oxidizing

action on the ascorbic acid (Dragan et al., 2008, Dinesh et al., 2015, Nielsen, 2017).

The method is based on the titration of the ascorbic acid in the vegetable extracts, with the redox indicator 2,6-dichloroindophenol, until it displays a pink colour which is persistent for 5 seconds (Nielsen, 2017).

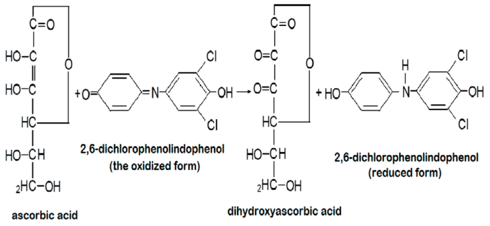

Figure 1. The reaction between ascorbic acid and 2,6-dichlorophenolindophenol

In the determination of vitamin C from samples, we used the method described by Dragan et al (2008) and Nielsen's readapted method (Nielsen, 2017).

Macronutrients composition

Both plants (soybeans and almond kernels) and plant milk samples were analysed by various methods to determine their nutritional and chemical composition (lipids, proteins, ash and carbohydrates). The crude protein content (Nx6.25) found in the samples was estimated by the macro-Kjeldahl method. The crude fats were determined by extraction with petroleum ether from a known weight of dried and grinded grains, using a Soxhlet extractor. For plant milk, we applied the same evaluation methods as for milk. We used the MilkoScan S54B device. The amount of carbohydrates was calculated by differences, according to the following equation:

$$Carbohidrates\ (\%) = 100 - [\text{Proteins } (\%) + \text{Lipids } (\%) + \text{Moisture } (\%) + Ash\ (\%)]$$

The energy values of samples formulas was calculated according to the following equation:

$$Total\ Energy\ (kcal/100g) = 4 \times [\text{Proteins } (\%) + \text{Carbohydrates } (\%)] + 9 \times [\text{Lipids } (\%)]$$

Sensory evaluation

For sensory evaluation were selected thirty panellists (15 females, and 15 males, aged between 19 and 22 years) to evaluate the aroma, colour, taste, smell, consistency and appearance of samples. This group was recruited from students of the Faculty of Food Science (University of Life Science "King Mihai I" from Timisoara) based on their health condition and consumption frequency of plant protein-based products. The samples (40 mL) were served cold (15 ± 2°C) in 50 mL plastic cups codified with three-digit random numbers (each sample being three times given for tasting, without the panelists' awareness regarding this aspect). Mineral water at room temperature and saltwater biscuits were served to allow the assessors to cleanse their palates between samples. Each consumer evaluated the four samples (SM, AM, A and B) in a single session. The sensorial properties were evaluated by a hedonic scale test with a 5-point (1 = dislike extremely, 3 = neither like nor dislike, 5 = like extremely) at 25°C.

Statistical analysis

All determinations were performed in triplicate, calculating their arithmetic mean of three separate determinations. The data were statistically analyzed using the program Microsoft Excel.

RESULTS AND DISCUSSIONS

Rheological analyses

Flowing is a continuous deformation of fluid, which takes place when the resultant of forces acting on the fluid is not zero.

Pascal's fluid is not viscous, thus being an ideal fluid which cannot be encountered among real fluids.

From a historical point of view, the classic theory of fluid dynamics has developed based on the theoretical studies conducted by Pascal, Bernoulli and Euler on a fluid that lacks viscosity - the ideal fluid or Pascal's fluid.

Real fluids are those that resist deformation and flowing, due to the friction forces between the layers. The intensity of these forces is expressed in the dynamic viscosity of fluids, thus leading to the conclusion that real fluids have viscosity.

In many fluids, viscosity depends only on the fluid's state parameters and not on the parameters related to the forces acting on the fluid (the frictional force and the frictional speed). These fluids are named Newtonian fluids (Mateescu, 2008, Yao et al., 2022).
The fluids that show a uniform flow behaviour index - as is the case of Newtonian fluids - but also show flow tension (τo > 0) are called plastic fluids or Bingham plastic. The behaviour of such a material is similar to the behaviour of an elastic solid when the tension is below the value of flow tension/effort τo.
From a rheological point of view, fluids can be studied if they are subject to a continuous friction at a constant speed. Ideally, this friction can be understood by the image of 2 parallel plates, at a certain distance from each other, having a real fluid within the space between the plates. If an external force is applied on the superior plate, it will move in the action direction of the force with a constant speed which depends on the value of the force (Mateescu, 2008; Depypere et al., 2009; Yao et al., 2022). The inferior plate is fixed, not mobile. The speed of the superior plate can be defined as an infinitesimal variation of the position within an extremely short time frame.The force with a parallel action to the superior plate will induce a friction force on the superior plate, which can be considered a layer of fluid of an infinitesimal thickness. Due to the adhesion forces between the fluid and the solid, the layer of fluid adjacent to the superior plate will move with the plate, at a speed equal to the plate's speed. Due to the cohesive molecular forces, this particular layer will put into motion the inferior neighbouring layer, however at a slower speed, and so forth, so that the motion is transmitted gradually to the entire mass of the fluid located between the two plates.
Since there is no gliding on the solid margin, the layer of fluid adjacent to the inferior plate has a zero speed. The rheological behaviour of alimentary fluids depends on their composition and on the temperature. (Mateescu, 2008, Yao et al., 2022).
Their flow behaviour is also influenced by the shear speed (velocity), the shear duration and the pre-shearing time. Recording of the fluids rheograms is a common way of obtaining a rheological characterisation of an alimentary fluid, as complete as possible. Rheograms express the dependence of the shear stress on the shear speed (Mateescu, 2008; Depypere et al., 2009; Yao et al., 2022).
Plant bazed beverages has been characterized from a rheological point of view at temperatures ranging between 5-40°C, the interval for temperature rise being of 5°C.
Experimental rheograms for homemade soymilk (obtained at room temperature) shown in Figure 2 as linear dependences σ = f (γ), (σ-shear stress, γ-shear speed). Row homemade soymilk behaves as a Newtonian fluid as well as soymilk prepared by boiling, which was described in a previous work (Rotariu et al., 2023).

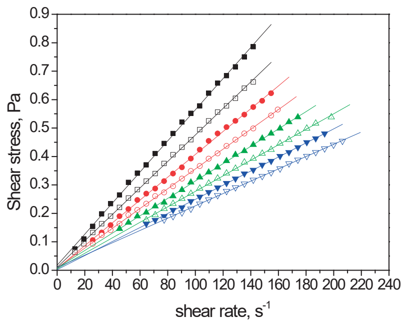

Figure 2. Rheograms for homemade raw soymilk – obtained at room temperature (■ – 5ºC; □ – 10ºC; ● – 15ºC; ○ – 20ºC; ▲- 25ºC; Δ – 30ºC; ▼– 35ºC ▽ – 40ºC; continous lines – calculated rheograms)

The characterization of boiled soybean milk and of purchased soybean milk was presented in a previous paper (Rotariu et al., 2023).
Table 2 indicates the viscosity values for all the eight evaluated temperatures. It can be noticed that this rheological characteristic is influenced by temperature, thus there is a decrease of viscosity levels as the temperature increases.

Table 2. The influence of temperature on the soybean milk viscosity

Temperature ºC	Raw soybean milk	
	Viscosity mPa s	SD
5	5.47	0.04
10	4.65	0.03
15	4.01	0.03
20	3.48	0.02
25	3.05	0.02
30	2.73	0.02
35	2.48	0.01
40	2.16	0.01

Note: SD - Standard deviation

The influence of temperature on viscosity, shown in Figure 3, displays a linear decrease in viscosity as temperature rises. Consequently, from a rheological point of view soymilk behaves as a Newtonian fluid.

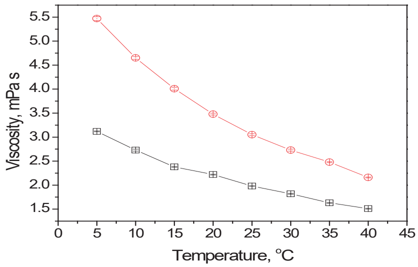

Figure 3. The influence of temperature on viscosity in soymilk (□ – homemade soymilk obtained by boiling); ○ – raw homemade soymilk)

Raw almond milk was evaluated rheologically at temperatures ranging between 5-35°C, the interval of temperature rise being of 5°C.
The experimental rheograms for raw almond milk are graphically expressed in Figure 4, in the form of variations σ = f (γ) nonlinear, (σ-shear stress, γ-shear velocity).

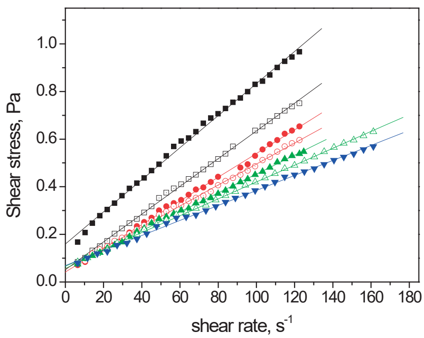

Figure 4. Raw almond milk rheograms (■ – 5°C; □ – 10°C; ● – 15°C; ○ – 20°C; ▲ - 25°C; Δ – 30°C; ▼ – 35°C; continuous lines – calculated rheograms)

The experimental rheograms graphically displayed in Figure 4 are in the form of variations σ = f (γ) linear, (σ - shear stress, γ - shear velocity). The non-linear variations suggest a non-Newtonian pattern of the fluid (raw almond milk), i.e. a Bingham character. (Mateescu, 2008; Yao et al., 2022). By non-linear regression, we determined the values of rheological properties for almond milk. The most appropriate model to apply in the case of this particular fluid is the Herschel-Bulkley model. Almond milk shows the behaviour of a Bingham fluid. Peanut milk had a similar character in the case of the studies by Yao et al. (2022).

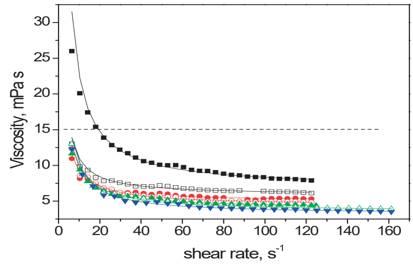

Figure 5. The influence of shear velocity on viscosity in raw almond milk (■ – 5°C; □ – 10°C; ● – 15°C; ○ – 20°C; ▲ - 25°C; Δ – 30°C; ▼ – 35°C; continuous lines – calculated viscosities; the dotted straight line at 15 mPa s represents the upper limit of the ordinate in this figure)

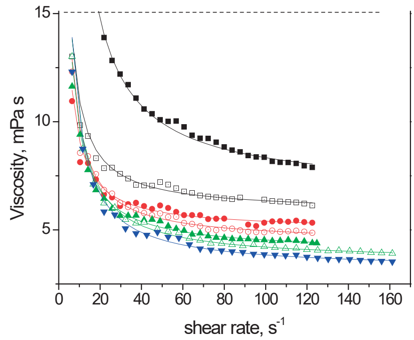

Figure 6. The influence of shear velocity on viscosity, for raw almond milk, in the range of viscosities below 15 mPa s (■ – 5°C; □ – 10°C; ● – 15°C; ○ – 20°C; ▲ - 25°C; Δ – 30°C; ▼ – 35°C; continuous lines – calculated viscosities)

Table 3 shows values for the flow effort and the plastic viscosity, at all seven studied temperatures. It can be noted that these rheological properties vary in relation to temperature; the flow effort reduces with the rise in temperature, and the plastic viscosity also decreases with the rise in temperature. This phenomenon is visible in Figure 7, which shows a pronounced decrease of viscosity when the shear velocity is higher.

Table 3. The rheological characteristics of raw almond milk, calculated for its behaviour as a Bingham fluid

Temperature, °C	Flow effort, mPa	SD	Plastic viscosity, mPa s	SD
5	159.79	5.15	6.74	0.07
10	51.70	2.61	5.83	0.04
15	41.69	3.04	4.98	0.04
20	54.64	2.36	4.41	0.03
25	56.60	1.99	3.97	0.03
30	66.25	1.70	3.54	0.02
35	69.30	2.50	3.15	0.03

Note: SD - Standard deviation

The influence of temperature on the values of rheological properties is described graphically in Figure 7 (for the flow effort) and Figure 8 (for the plastic viscosity).

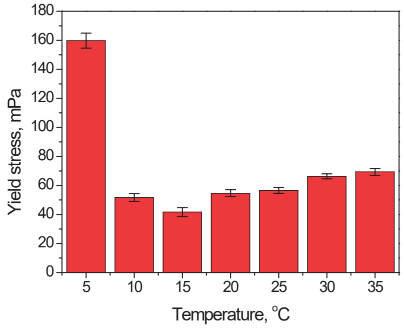

Figure 7. The influence of temperature on flow effort, in raw almond milk (the standard deviation is marked as ±Y bars)

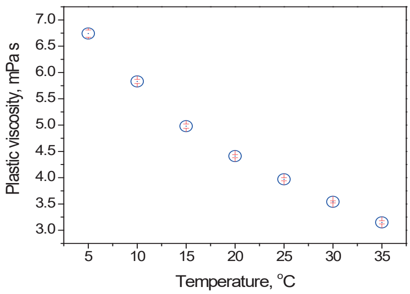

Figure 8. The influence of temperature on plastic viscosity, in raw almond milk (the standard deviation, in red, is marked as ±Y bars)

The experimental data indicate that the influence of temperature on the flow effort, in almond milk - Figure 7 - manifests as follows: the temperature rise determines the decline of the flow effort, up to 15ºC temperature, and afterwards an increase of the flow effort takes place. On the contrary, the plastic viscosity of raw almond milk (Figure 8) shows a decrease, as the temperature rises.

Physicochemical and nutritional properties of soybean and almond kernels

The results of the physical, chemical and nutritional characterization of fruits (soybean and almond kernels) used for preparation plant milk are shown in Table 4.

Table 4. Physical and chemical characterization of the soybean and almond kernels

Parameters	Soybean	Almond kernels
Fruit weight, g	0.22	1.09
Moisture (g/100 g)	10.15	17.93
TDW (g/100 g)	88.98	82.07
Protein (g/100 g)	36,09	26.47
Lipids (g/100 g)	17.01	47.08
Carbohydrates (g/100 g)	31.64	5.61
Ash (g/100 g)	5.11	2.91
Energy (kcal/100 g)	423.96	552.04
TDW: Total dry weight, TSS: total soluble solids		

Nutritionally speaking, soybean is a protein-rich product. This fact may also be observed in the soybeans that we use for milk preparation. Amongst the macronutrients, the protein content of soybeans is the highest (36.09 g/100 g), followed by carbohydrates (31.64 g/100 g). In almond kernels, lipid content is the highest (47.08 g/100 g), followed by protein (26.47 g/100 g), and carbohydrates have the lowest content.

Physicochemical and nutritional properties of plant-based milk

The results of the basic nutritional and physico-chemical characterization plan-based milk beverages are shown in Table 5.

Table 5. A nutritional and physico-chemical characterization of the plant milk beverages

Parameters	A	AM	B	SM
pH	6.18	6.02	6.87	6.18
TSS (ºBrix)	8.9	7.6	9.2	4.9
Salinity	8.5	6.4	4.3	3.9
Moisture (g/100 g)	95,57	93.21	91.9	92.3
TDW (g/100 g)	4.43	6.79	8.1	7.77
Ash (g/100 g)	0.4	0.63	0.43	0.37
Carbohydrates (g/100 g)	2.39	2.08	2.54	3.3
Protein (g/100 g)	0.45	2.3	3.33	2.9
Lipids (g/100 g)	1.19	1.78	1.8	1.2
Energy (kcal/100 g)	22.07	34.14	39.56	35.6

From the data displayed in Table 5 it can see that the value of total soluble solids content (TSS content - brix degree) was prominent in B sample (9.2) and the lower value (4.9) measured for SM sample (homemade soymilk). The brix values are higher for raw almond milk (7.6) as compared to soymilk. As regards the pH, one may notice that the values range within the 6.18-6.87 frame. The highest pH value belongs to B sample, while the lowest is recorded for the milk prepared by us. Regarding salinity, the highest value was also found in the almond milk purchased from supermarkets and the lowest value was found in homemade soymilk. Plant-based milk prepared at home has lower values than that purchased from stores. These low values can be explained and taking into account the fact that the preparation of milk was made only from fruit and water, no additives were used.

The data listed in Table 5 show that the lowest macronutrient content is found in soymilk purchased for supermarket (A sample). The differences between these A and AM samples can be explained by the fact that various soybeans quantities were used in proportion with the water added for the milk preparation. Likewise, for the improvement of milk sensory qualities other products are also introduced along with the "fruits" (soybeans and almond kernels), with specific macronutrient content.

The energetic value of the studied plant milk ranges between 22 and 40 (kcal/100 g). The lowest energetic value is found in almond milk purchased for supermarkets.

The energetic value of the studied homemade plant milk is around of 35 kcal/100 g (34.14 for AM and 35.6 for SM).

TSS values for soymilk are in the range from 8.1 to 10.10°Brix, reported by Terharg et al. (2013) and Micula et al. (2016; 2023), lower than those reported by Villegas, Carbonell and Costell (2011) for Spanish vanilla-flavored soy beverages (10.5 to 18.3°Brix). Callou et al. (2010) reported higher protein contents for soy beverages commercialized in the Brazilian market. Moore et al. (2023) reported, for soymilk and almond milk, close values of the content in macronutrients (Proteins - P, Lipids - L, carbohydrates - CH) and total mineral substances (ash). On the other hand, Milovanovic et al. (2023) reported lower values than the vegetable milk values studied by us.

This result confirms that the presence of aroma and flavour, darker colour, and different viscosity are important attributes in the acceptance of soymilk plain beverages. Villegas et al. (2011) have suggested that it is possible to increase the acceptability of vanilla-flavoured soy beverages by increasing their sweetness, viscosity, and intensity of vanilla flavour.

Sensory evaluation of plant based beverages

The results of sensorial assay are presented in Table 6 and Figure 9. Where, A and B sample are commercial products (described in Table 1).

Table 6. The sensorial and physico-chemical characterization of the soymilk sample

Parameters	SM	AM	A	B
Appearance	2.96	3	3.45	2.95
Colour	3.03	3.6	3.3	3.25
Consistance	2.59	3.65	4.6	4.06
Taste	2.05	3.59	4.7	2.95
Smell	2.46	3.65	3.75	3
Aroma	2.1	4	3.96	3.06

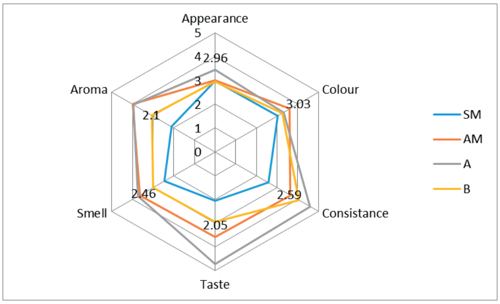

Figure 9. The sensorial characterization of the plant milk sample

One of the objectives of our research was to relate the physical and chemical characteristics of plant milk beverages to its sensory acceptance. From a sensorial point of view, the soymilk and almond milk samples from markets were well appreciated.

As expected, the least appreciated was the beverages prepared by us, since it does not contain any addition meant to improve its sensorial qualities, especially since it was prepared at room temperature, without other subsequent operations on the grains (such as roasting or germinating the grains, or others..

The least appreciated was the soymilk prepared by us,
The lowest score was registered on the taste level (2.05) and the highest score was recorded by the A (almond milk) sample, for the taste, (4.7).
The mostly appreciated sample from a sensorial point of view (B - soymilk and A - almond milk), displays the highest score for TSS (total soluble solids content.
Among the drinks prepared by us, the most appreciated was the almond milk, it received a score higher than 3, on the other hand, the soy milk was the least appreciated, the score obtained did not exceed 3, and for the taste, it was the lowest score of all assessments made.
On the aroma, the lowest level score was 2.1 for soymilk prepared by us and the highest score was 4 recorded by the AM sample (prepared by us) as well appreciated as the marketed one. The milk prepared by us can improve its sensorial qualities by adding some components (such as salt, inverted sugar, etc.), or in the preparation process to include other stages of 'grain' preparation, such as roasting etc. It was observed that the drinks prepared by Yao et al. (2022) were better appreciated, and the grains (that were used in the preparation of milk.) were roasted This fact can also be explained by these pretreatments performed on the grains.

CONCLUSIONS

This present study was designed to provide the scientific basis for developing plant-based milk by evaluating the physiochemical, rheological and sensory characteristics of two types of plant-based kinds of milk.
Soy beans and almond kernels are products with rich macronutrient content. Soybeans represent a significant source of protein, having beneficial properties for health. The moisture content in soybeans is relatively low. Almonds contain a large amount of soluble sugars, proteins, lipids, minerals, fibres and antioxidants.
Unlike milk of animal origin, plant milk contains neither cholesterol nor lactose, being an alternative food used by people who are lactose intolerant or have allergies to gluten and casein. It is also accepted by vegans, vegetarians and fasting people.
Homemade almond milk prepared by us behaves as a Bingham fluid. Non-linear dependences in the form of variations σ = f (γ) nonlinear, (σ- shear stress, γ-shear velocity) suggest a non-Newtonian pattern of the fluid, a Bingham character. The most appropriate model to apply in the case of this particular fluid is the Herschel-Bulkley model. Soymilk - both the homemade one obtained at room temperature and one obtained by boiling - behaves as a Newtonian fluid. Linear dependences suggest a Newtonian character of the fluid (the soymilk).
The nutritional qualities of plant-based milk (almond and soy milk, respectively), are due to the high content of valuable nutrients for the human body, contributing to the energisation, vitaminization and mineralisation of the body.
The physical-chemical analyses of these raw-vegan preparations make it possible to know the intake within the recommended daily diet.
The presence of flavour, darker colour, and different viscosity degrees are important attributes in the acceptance of plant-based milk beverages.
The preparation recipe of homemade plant-based milk must be improved regarding all the physico-chemical and sensorial characteristics.
Therefore, future researchers or manufacturers could base the results obtained from this study and add food flavourings to make new kinds of plant-based milk alternatives more palatable to consumers.

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OVERVIEW OF BIOACTIVE COMPOUNDS, BIOLOGICAL PROPERTIES AND THERAPEUTIC EFFECTS OF *PLECTRANTHUS AMBOINICUS*

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Abstract

Plectranthus amboinicus (Lour.) Spreng is a perennial plant belonging to the Lamiaceae family which is found naturally in the tropics and warm regions of Australia, Asia and Africa. This plant has therapeutic properties (antioxidant, anti-inflammatory, antimicrobial, antitumor, antiepileptic and analgesic activities) attributed to its phytochemical compounds (76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes), which are highly valued in the pharmaceutical industry. P. amboinicus is widely used in traditional medicine to treat respiratory, cardiovascular, digestive, urinary and skin conditions such as cold, cough, fever, asthma, constipation, headache and skin diseases. In recent years, due to the increased interest in herbal treatments, numerous research studies have been conducted to document the traditional uses of P. amboinicus and to find new biological effects of this plant. This review provides comprehensive information on the biological properties and bioactive compounds responsible for the therapeutic effects of P. amboinicus.

Key words: *Indian borage, medicinal plant, phytochemicals, biological activities, pharmacological properties.*

INTRODUCTION

According to the World Health Organization, about 80% of people worldwide use traditional herbal medicines to treat various diseases and help maintain health due to their low cost and negligible side effects compared to allopathic medicines (Sandhya et al., 2011; Swamy et al., 2011; Swamy & Sinniah, 2015; Swamy et al., 2015). The widespread use of natural products has led to increased global demand for medicinal plants. Current research aims to explore and exploit new plant species for their medicinal properties (Kumara et al., 2012; Mohanty et al., 2014; Swamy et al., 2015). Several genera of the *Lamiaceae* family, such as *Plectranthus*, contain plant species that have important pharmacological properties. Over 300 species of *Plectranthus* are found naturally in tropical and warm regions of Australia, Asia and Africa (Retief, 2000). More than 85% of the literature on *Plectranthus* refers to the therapeutic value of the species of this genus, followed by its nutritional and horticultural properties attributed to its aromatic nature and ability to produce essential oil (Alasbahi & Melzig, 2010; Grayer et al., 2010). *Plectranthus amboinicus* (Lour.) Spreng is one of the most documented species of the *Plectranthus* genus. *P. amboinicus*, commonly known as Indian borage, is a plant renowned for its medicinal properties and distinct oregano-like aroma and smell (Lukhoba et al., 2006). This plant is widely used both in folk medicine and for culinary purposes. This is mainly due to the high amounts of bioactive compounds in the essential oil of its leaves, such as Carvacrol (Castillo & Gonzalez, 1999), Thymol (Singh et al., 2002), β-Caryophyllene, α-Humulene, γ-Terpinene, p-Cymene, α-Terpineol and β-Selinene (Murthy et al., 2009; Senthilkumar & Venkatesalu, 2010). These biochemical constituents exhibit several biological activities (antioxidant, anti-inflammatory, antimicrobial, antitumor, antiepileptic and analgesic activities) due to which this plant is widely used in traditional medicine to treat conditions such as cold, cough, fever, asthma, constipation, headache and skin diseases (Bhatt & Negi, 2012; Gonçalves et al., 2012; Arumugam et al., 2016). This review provides comprehensive information on the bioactive compounds, biological properties and therapeutic effects of *P. amboinicus*.

BIOACTIVE COMPOUNDS OF *P. AMBOINICUS*

The specialized literature highlighted the presence in the phytochemical composition of *P. amboinicus* of several volatiles and non-volatile compounds belonging to different classes of phytochemicals.

The essential oil of *P. amboinicus* contains 76 volatile compounds belonging to the classes of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The two major compounds of essential oil are Carvacrol and Thymol (Lukhoba et al., 2006; Can Baser, 2008; Roshan et al., 2010; Khare et al., 2011).

P. amboinicus also contains 30 non-volatile compounds belonging to the classes of phenolic acids, flavonoids, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes and esters (Khare et al., 2011; El-hawary et al., 2012a).

However, the chemical profile varies depending on various factors such as geographical area, climate, harvesting stage of plant material and extraction method (Swamy & Sinniah, 2015).

Tables 1 and 2 show the main volatile and non-volatile compounds of *P. amboinicus*.

Table 1. The main volatile compounds of *P. amboinicus*

Phytochemical class	Compound name	Plant origin	Plant part	References
Monoterpene hydrocarbons	δ-3-Carene	India, Malaysia, Mauritius, Morocco	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Hassani et al. (2012) Erny Sabrina et al. (2014)
	p-Cymene	Brazil, Cambodia, India, Malaysia, Venezuela	Aerial part, leaf	Mallavarapu et al. (1999) Singh et al. (2002) Koba et al. (2007) Murthy et al. (2009) Velasco et al. (2009) Da Costa et al. (2010) Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Kweka et al. (2012) Manjamalai & Grace (2012) Erny Sabrina et al. (2014)
	Limonene	India, Mauritius	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999)
	β-Myrcene	Cambodia, India, Venezuela	Leaf	Mallavarapu et al. (1999) Koba et al. (2007) Murthy et al. (2009) Velasco et al. (2009) Manjamalai & Grace (2012)
	Ocimene	Morocco	Leaf	Mallavarapu et al. (1999) Hassani et al. (2012)
	α-Phellandrene	India, Comoros, Mauritius, Venezuela	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Velasco et al. (2009) Hassani et al. (2012)
	β-Phellandrene	India	Leaf	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	α-Pinene	Cambodia, India	Leaf	Mallavarapu et al. (1999) Koba et al. (2007)
	β-Pinene	India	Leaf	Mallavarapu et al. (1999)
	Sabinene	Cambodia, India, Morocco	Leaf	Mallavarapu et al. (1999) Koba et al. (2007) Hassani et al. (2012)
	α-Terpinene	India, Mauritius	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)

Phytochemical class	Compound name	Plant origin	Plant part	References
				Manjamalai & Grace (2012) Tewari et al. (2012)
	γ-Terpinene	Brazil, Cambodia, India, Malaysia, Mauritius	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Koba et al. (2007) Murthy et al. (2009) Da Costa et al. (2010) Senthilkumar & Venkatesalu (2010) Kweka et al. (2012) Manjamalai & Grace (2012) Erny Sabrina et al. (2014)
	α-Terpinolene	Brazil, Morocco	Leaf	Da Costa et al. (2010) Hassani et al. (2012)
	α-Thujene	Comoros, India, Venezuela	Leaf	Mallavarapu et al. (1999) Velasco et al. (2009) Senthilkumar & Venkatesalu (2010) Hassani et al. (2012) Kweka et al. (2012)
Oxygenated monoterpenes	Camphor	Comoros, Malaysia, Mauritius	Leaf	Gurib-Fakim et al. (1995) Hassani et al. (2012) Erny Sabrina et al. (2014)
	Carvacrol	Cambodia, India, Malaysia, Mauritius, Venezuela	Aerial part, leaf, flower	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Singh et al. (2002) Mangathayaru et al. (2005) Murthy et al. (2009) Velasco et al. (2009) Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Kweka et al. (2012) Manjamalai & Grace (2012) Tewari et al. (2012) Asiimwe et al. (2014) Erny Sabrina et al. (2014)
	Carvone	India	Leaf	Mallavarapu et al. (1999)
	1,8-Cineole	India	Leaf	Mallavarapu et al. (1999) Singh et al. (2002) Knab et al. (2008) Rout et al. (2012)
	Eugenol	Cambodia, India	Leaf	Mallavarapu et al. (1999) Koba et al. (2007) Uma et al. (2011) Tewari et al. (2012)
	Geraniol	Mauritius	Leaf	Gurib-Fakim et al. (1995)
	Linalool	Comoros, Mauritius	Leaf	Gurib-Fakim et al. (1995) Hassani et al. (2012)
	Methyl carvacrol	India	Leaf	Mallavarapu et al. (1999)
	Methyl eugenol	Cambodia	Leaf	Mallavarapu et al. (1999) Koba et al. (2007)
	α-Terpineol	Comoros, India, Venezuela	Leaf	Mallavarapu et al. (1999) Velasco et al. (2009) Hassani et al. (2012)
	Terpinen-4-ol	Brazil, India, Mauritius	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Singh et al. (2002) Murthy et al. (2009) Da Costa et al. (2010) Hassani et al. (2012) Tewari et al. (2012)
	Thymol	Brazil, Cambodia, India, Venezuela	Aerial part, leaf	Mallavarapu et al. (1999) Singh et al. (2002) Roja et al. (2006) Murthy et al. (2009) Velasco et al. (2009) Da Costa et al. (2010) Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Uma et al. (2011) Kweka et al. (2012) Manjamalai & Grace (2012) Tewari et al. (2012)
	Thymol methyl ether	Brazil	Leaf	Da Costa et al. (2010)
Sesquiterpene hydrocarbons	α-Amorphene	Cambodia	Leaf	Koba et al. (2007)
	Aromadendrene	Brazil, India	Leaf	Da Costa et al. (2010) Manjamalai & Grace (2012)

Phytochemical class	Compound name	Plant origin	Plant part	References
	trans-α-Bergamotene	Brazil, Comoros, India, Venezuela	Aerial part, leaf, flower	Murthy et al. (2009) Velasco et al. (2009) Da Costa et al. (2010) Joshi et al. (2011) Hassani et al. (2012)
	trans-β-Bergamotene	Cambodia	Leaf	Koba et al. (2007)
	γ-Cadinene	Cambodia	Leaf	Koba et al. (2007)
	δ-Cadinene	Cambodia, India	Leaf	Mallavarapu et al. (1999) Murthy et al. (2009)
	α-Calacorene	India	Aerial part	Joshi et al. (2011)
	cis-Calamenene	Cambodia	Leaf	Koba et al. (2007)
	β-Caryophyllene	Brazil, India, Venezuela	Leaf, flower	Mallavarapu et al. (1999) Murthy et al. (2009) Velasco et al. (2009) Da Costa et al. (2010) Joshi et al. (2011) Hassani et al. (2012) Manjamalai & Grace (2012)
	γ-Caryophyllene	India	Leaf	Mangathayaru et al. (2005)
	α-Copaene	Comoros, India	Leaf	Mallavarapu et al. (1999) Hassani et al. (2012)
	α-Cubebene	India	Aerial part, leaf	Murthy et al. (2009) Joshi et al. (2011)
	(E,Z)-α-Farnesene	France	Leaf	Prudent et al. (1995) Mallavarapu et al. (1999)
	Germacrene D	Cambodia	Leaf	Koba et al. (2007)
	γ-Gurjunene	India	Aerial part	Joshi et al. (2011)
	Humulene	Brazil, Cambodia, India, Morocco, Venezuela	Aerial part, leaf	Roja et al. (2006) Koba et al. (2007) Velasco et al. (2009) Da Costa et al. (2010) Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Hassani et al. (2012) Kweka et al. (2012)
	α-Muurolene	Cambodia, France, Mauritius	Leaf	Gurib-Fakim et al. (1995) Prudent et al. (1995) Koba et al. (2007)
	Patchoulene	India, Mauritius	Leaf	Gurib-Fakim et al. (1995) Mangathayaru et al. (2005)
	β-Selinene	Comoros, India	Leaf	Senthilkumar & Venkatesalu (2010) Uma et al. (2011) Hassani et al. (2012) Kweka et al. (2012)
	β-Sesquiphellandrene	Cambodia	Leaf	Prudent et al. (1995)
Oxygenated sesquiterpenes	Caryophyllene oxide	Cambodia, India, Venezuela	Aerial part, leaf	Mallavarapu et al. (1999) Koba et al. (2007) Murthy et al. (2009) Velasco et al. (2009) Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Uma et al. (2011) Kweka et al. (2012) Tewari et al. (2012)
	β-Cedrene epoxide	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	β-Copaen-4-α-ol	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	1-Epi-cubenol	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	β-Eudesmol	India	Leaf	Mallavarapu et al. (1999)
	β-Himachalene oxide	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	Humulene oxide	India	Leaf	Mallavarapu et al. (1999)
	Spathulenol	India	Leaf	Singh et al. (2002) Tewari et al. (2012)
Others (alcohols, aldehydes, esters, fatty acids, phenylpropanoids, terpenes)	1,2-Benzenediol 4-(1,1 dimethylethyl)	India	Leaf	Uma et al. (2011)
	Chavicol	India	Leaf	Rout et al. (2012)
	Methyl chavicol	India	Aerial part	Senthilkumar & Venkatesalu (2010) Joshi et al. (2011) Kweka et al. (2012)
	α-Corocalene	India	Aerial part	Joshi et al. (2011)
	Dihydro carveol	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	Durohydroquinone	India	Leaf	Uma et al. (2011)

Phytochemical class	Compound name	Plant origin	Plant part	References
	1,4 Eicosadiene	India	Leaf	Uma et al. (2011)
	Ethyl Salicylate	India	Leaf	Rout et al. (2012)
	(Z)-1,3-Hexadiene	France	Leaf	Prudent et al. (1995)
	(Z)-3-Hexen-1-ol	France	Leaf	Prudent et al. (1995) Mallavarapu et al. (1999)
	Methyl octanoate	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	1-Octen-3-ol	India, Mauritius, Venezuela	Leaf	Gurib-Fakim et al. (1995) Mallavarapu et al. (1999) Velasco et al. (2009) Tewari et al. (2012)
	Oleic acid	India	Leaf	Uma et al. (2011)
	2-Phenyl ethyl tiglate	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	Phytol	India	Leaf	Uma et al. (2011)
	Squalene	India	Leaf	Uma et al. (2011)
	Tetradecanal	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	India	Leaf	Uma et al. (2011)
	Thymol acetate	India	Leaf	Mallavarapu et al. (1999) Tewari et al. (2012)
	Trans-sabinene hydrate	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)
	Undecanal	India	Aerial part	Senthilkumar & Venkatesalu (2010) Kweka et al. (2012)

Table 2. The main non-volatile compounds of *P. amboinicus*

Phytochemical class	Compound name	Plant origin	Plant part	Extract type	References
Phenolic acids	Caffeic acid	Egypt, India	Leaf, stem, root	Methanol extract	El-hawary et al. (2012a) Bhatt et al. (2013)
	Gallic acid	India	Stem	Methanol extract	Bhatt et al. (2013)
	p-Coumaric acid	Egypt, India	Leaf, stem, root	Methanol extract, ethyl acetate fraction	El-hawary et al. (2012a) Bhatt et al. (2013)
	Rosmarinic acid	Egypt, India, Thailand	Leaf, stem, root	Methanol extract, ethyl acetate fraction	El-hawary et al. (2012a) Bhatt et al. (2013) Chen et al. (2014)
	Salvianolic acid A	Thailand	Aerial part	Water extract	Chen et al. (2014)
	Shimobashiric acid	Thailand	Aerial part	Water extract	Chen et al. (2014)
Flavonoids	Chrysoeriol	Egypt, Philippines	Leaf, stem, root	Chloroform extract, ethyl acetate fraction	Ragasa et al. (1999) El-hawary et al. (2012a)
	Cirsimaritin	Philippines	Leaf	Chloroform extract	Ragasa et al. (1999)
	Eriodictyol	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	Luteolin	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	Rutin	India	Stem	Methanol extract	Bhatt et al. (2013)
	Salvigenin	Philippines	Leaf	Chloroform extract	Ragasa et al. (1999)
	Thymoquinone	Thailand	Aerial part	Water extract	Chen et al. (2014)
	Quercetin	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a) Bhatt et al. (2013)
	5,4'-Dihydroxy-6,7-dimethoxy flavone	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	5,4'-Dihydroxy-3,7-dimethoxy flavone	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	5-O-Methyl-luteolin	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	3,5,7,3',4'-Pentahydroxy flavanone	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)
	4',5,7-Trihydroxyflavone (apigenin)	Egypt	Leaf, stem, root	Ethyl acetate fraction	El-hawary et al. (2012a)

BIOLOGICAL PROPERTIES AND THERAPEUTIC EFFECTS OF *P. AMBOINICUS*

In folk medicine, formulations from *P. amboinicus* are used in the treatment of various ailments, such as cough, fever, nasal congestion, headaches, oral diseases, colic, indigestion, hepatopathy, convulsions, epilepsy, gallstones, nephrolithiasis or rheumatism. The fresh leaves are also used to soothe burns or animal and insect bites and to treat skin wounds and inflammation. In addition, due to the high content of minerals (calcium, iron, magnesium, potassium, zinc), this plant can be consumed as food to maintain health or in combination with probiotic products to restore normal intestinal microflora (Arumugam et al., 2016).

The long-term use of *P. amboinicus* in naturopathic medicine in many countries of the world is demonstrated by numerous scientific studies reporting strong antimicrobial, antioxidant, anti-inflammatory, antitumor, analgesic, larvicidal, antileishmanial, anthelmintic, wound healing, lactogenic, antiepileptic and anticonvulsant properties of this plant species. It has also been found that the essential oil from this plant can be used as a natural repellent or agent to control mosquito populations (Arumugam et al., 2016).

However, due to the high variability of its chemical composition, further research on the biological activities and potential pharmacological applications of this species is needed (Arumugam et al., 2016).

Table 3 shows in detail the biological properties and therapeutic effects of *P. amboinicus*.

Table 3. Biological properties and therapeutic effects of *P. amboinicus*

Biological properties	Plant part / Extract type	Therapeutic effects	References
Antibacterial properties	Leaf extract / Decoction / Essential oil	Antibacterial properties against *Bacillus cereus, B. subtilis, Enterococcus faecalis, E. faecium, Escherichia coli, Klebsiella pneumoniae, Lactobacillus, Mycobacterium tuberculosis*, *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella bayedir, Staphylococcus aureus, Streptococcus mutans, Vibrio cholerae* and *Yersinia enterocolitica*	Frame et al. (1998) Akagawa et al. (2003) Oliveira et al. (2007) Vermelho et al. (2007) Bhatt & Negi (2012) Bhatt et al. (2013) Majee et al. (2013) Oliveira et al. (2013) Muniandy et al. (2014) Aguiar et al. (2015) Shubha & Bhatt (2015) Vijayakumar et al. (2015) Manojkanna et al. (2017) Sireesha et al. (2017) Ismayil & Nimila (2019) Manojkanna et al. (2019) Sreelakshmy & Thangapandiyan (2019) Jena et al. (2023)
Antifungal properties	Leaf extract / Essential oil	Fungitoxic properties against *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. oryzae*, *Candida albicans*, *C. krusei*, *C. parapsilosis, C. stellatoidea, C. tropicalis*, *C. versatilis*, *Fusarium* sp., *Penicillium* sp. and *Saccharomyces cerevisiae*	Oliveira et al. (2007) Murthy et al. (2009) El-hawary et al. (2012b) Manjamalai et al. (2012) Majee et al. (2013) EL-Zefzafy et al. (2016) Manojkanna et al. (2017) Sivaranjani et al. (2019)
Antiviral properties	Ethanolic leaf extract	Antiviral properties against human immunodeficiency virus (HIV), herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV)	Hattori et al. (1995) Kusumoto et al. (1995) Ali et al. (1996) Asiimwe et al. (2014)
Antioxidant properties	Aqueous, ethanolic, methanolic, acetone and ethyl acetate leaf extract / Essential oil	Significant inhibition of DPPH free radical and hydroxyl radical formation; high nitric oxide scavenging, superoxide scavenging and ferrous ion chelating capacity	Kumaran & Karunakaran (2006) Khanum et al. (2011) Bhatt & Negi (2012) Manjamalai & Grace (2012) Gurning (2020) Terto et al. (2020) Puspitarini et al. (2023)
Anti-inflammatory properties	Aqueous, ethanolic, methanolic and hexanoic extract from the aerial part	Inhibitory effect on DNA binding activities; inhibitory activities on activator protein 1 (AP-1) and tumor necrosis factor alpha (TNF-α); positive effect on carrageenan-induced paw edema; increased IgG and IgM levels and lysozyme activity in rats	Gurgel et al. (2009) Chiu et al. (2012) Manjamalai & Grace (2012) Chen et al. (2014) Silitonga et al. (2015) Puspitarini et al. (2023)
Antitumor properties	Ethanolic, hexanoic and ethyl acetate leaf extract / Crude hydroalcoholic extract / Essential oil	Cytotoxic activity against Sarcoma 180 cell line (S-180), cervical adenocarcinoma cells (HeLa), breast cancer cell lines (MCF-7, MDA-MB-231, T-47D), colorectal cancer cell lines (HT-29, HCT-116), human lung cancer cell line (A549), oral cancer cell line (KB), Vero cells lines and Ehrlich ascites carcinoma tumors in mice	Gurgel et al. (2009) Hasibuan et al. (2013) Ramalakshmi et al. (2014) Rosidah & Hasibuan (2014) Hasibuan & Rosidah (2015) Hasibuan et al. (2015) Thirugnanasampandan et al. (2015) Hasibuan & Sumaiyah (2019) Caroline et al. (2023) Gupta et al. (2024) Mujamammi et al. (2024)
Analgesic properties	Aqueous,	Soothing effect on headaches, backaches and	Chiu et al. (2012)

Biological properties	Plant part / Extract type	Therapeutic effects	References
	alcoholic and ethyl acetate leaf extract	musculoskeletal conditions such as neck and back pain	El-hawary et al. (2012b) Majee et al. (2013) Chen et al. (2014)
Larvicidal, antileishmanial and anthelmintic properties	Ethyl acetate leaf extract / Alcoholic extracts of leaves, stems and roots / Essential oil	Larvicidal properties against *Aedes aegypti* (the main vector of dengue, yellow fever and dengue haemorrhagic fever), *Anopheles stephensi* (malaria vector mosquitoes), *Anopheles gambiae, Culex quinquefasciatus* and *Culex tritaeniorhynchus*; antileishmanial activity against *Leishmania braziliensis*; anthelmintic activity against the intestinal parasite *Pheritima posthuman*	Senthilkumar & Venkatesalu (2010) Lima et al. (2011) Prasenjit et al. (2011) Verma et al. (2012) Baranitharan & Dhanasekaran (2014) Lima et al. (2014) Jayaraman et al. (2015) Vijayakumar et al. (2015) Gonçalves (2017)
Wound healing properties	Aqueous and ethanolic extract of leaves and roots / Leaf and root paste	Preventing or reducing the risk of wound infection and stimulating wound healing in diabetic mice by increasing collagen deposition and reducing wound epithelialization time; good wound healing properties of *P. amboinicus* and *Punica granatum* suspension	Warriner & Burrell (2005) Sunitha et al. (2010) Soni et al. (2011) Jain et al. (2012) Muniandy et al. (2014)
Lactogenic properties	Leaves	Increase in breast milk production in new mothers due to the high content of nutrients in the leaves, especially carotene and iron; increasing the mineral content of milk (iron, magnesium, potassium and zinc)	Silitonga et al. (2015)
Antiepileptic and anticonvulsant properties	Aqueous and alcoholic extract of leaves, stems and roots	Antiepileptic and anticonvulsant effects in the treatment of nervous disorders, including epilepsy and convulsions	Jain & Lata (1996) Castillo & Gonzalez (1999) Bhattacharjee & Majumder (2013)
Biological properties against respiratory diseases	Leaf extract / Decoction / Juice / Essential oil	Expectorant and bronchodilator properties in the treatment of flu, cough, bronchitis, asthma and sore throat, properties attributed mainly to the compounds carvacrol and thymol in the essential oil of the plant	Jain & Lata (1996) Castillo & Gonzalez (1999) Singh et al. (2002) Cano & Volpato (2004) Cartaxo et al. (2010)
Biological properties against digestive diseases	Leaf extract / Juice	Stimulating effect on the growth of *Lactobacillus plantarum*; inhibitory effect on the growth of *Escherichia coli*, *Salmonella typhimurium, Shigella* sp. and *Vibrio* sp.; prevents the formation of gases in the gastrointestinal tract and facilitates the elimination of gases; positive effect in treating diarrhea, constipation, dyspepsia and indigestion	Gurib-Fakim et al. (1995) Jain & Lata (1996) Ong & Nordiana (1999) Hassani et al. (2012) Shubha & Bhatt (2015) Sivaranjani et al. (2019) Leesombun et al. (2023)
Biological properties against cardiovascular diseases	Aqueous leaf extract	Positive inotropic activity in isolated frog heart; positive effect in the treatment of congestive heart failure	Hole et al. (2009)
Biological properties against genitourinary diseases	Aqueous, ethanolic and ethyl acetate leaf extract / Leaf decoction / Juice	Effective in treating urinary and kidney disorders and vaginal discharges; increased urine volume and the electrolyte concentration (Na, K and Cl ions) in male albino rats; dissolving crystals in the urinary tract; uric acid reducing activity; good results in cisplatin-induced nephropathies, increasing the expression of TGF-1β (transforming growth factor-1β) and thus inhibiting renal necrosis and cellular infiltration	Yoganarasimhan (2000) Jose & Janardhanan (2005) Palani et al. (2010) Patel et al. (2010) El-hawary et al. (2012b) Amarasiri et al. (2018) Sahrial & Solfaine (2019) Wang et al. (2023)
Biological properties against oral diseases	Essential oil	Antibacterial effect alone or combined with mouthwash, modulating the activity of the mouthwash against *Streptococcus mutans*, the main bacteria responsible for dental caries	Santos et al. (2016)
Biological properties against skin diseases	Leaf extract / Leaf juice / Paste / Ethanolic root extract ointment / Essential oil	Anti-dandruff effect due to the inhibitory activity on the development of dandruff-producing fungus *Malassezia furfur*; antiseptic properties on cuts; antipsoriasis effect; positive effect in the treatment of skin ulcerations caused by *Leishmania braziliensis*, skin allergies and skin burns acting as an antiseptic and promoting healing	França et al. (1996) Jain & Lata (1996) Harsha et al. (2003) Bhat et al. (2012) Selvakumar et al. (2012) Vijayalakshmi et al. (2019)
Biological properties against animal and insect bites	Leaves / Aqueous leaf extract	Effective as a poultice for scorpion and centipede bites and as an antidote for scorpion venom (*Heterometrus laoticus*)	Jain & Lata (1996) Uawonggul et al. (2006)
Biological properties against other diseases	Aqueous, ethanolic and acetonic leaf extract	Effective in treating infectious diseases such as fever, cholera and meningitis, and sensory disorders associated with eye and ear problems such as conjunctivitis and acute edematous otitis; hepatoprotective effect; photoprotective activity; antidiabetic activity by inhibiting alpha-amylase	Neuwinger (2000) Harsha et al. (2002) Patel (2011) Bole & Kumudini (2014) Terto et al. (2020) Silitonga et al. (2023)

CONCLUSIONS

P. amboinicus is a valuable aromatic medicinal plant rich in bioactive constituents with a wide range of biological properties. This plant is effective in treating cardiovascular, respiratory, skin, oral, urinary and digestive diseases. The important therapeutic effects of *P. amboinicus* confirm the possibility of its use in more cost-effective and safer natural drug formulations than allopathic drugs. However, future investigations should aim to isolate and identify new bioactive compounds from *P. amboinicus* and test their effectiveness in treating other human diseases. Further research is also needed on the toxicity of these isolated compounds and human safety aspects regarding the use of this plant. Therapeutic applications of *P. amboinicus* may also extend its use as a functional nutraceutical food.

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NATURA 2000 HABITATS FROM OLTENIA AFFECTED BY INVASIVE AND POTENTIALLY INVASIVE SPECIES (II)

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Abstract

The present paper is a complex segment of a study aimed at Natura 2000 habitats that are affected by invasive and potentially invasive plants. In the first paper, the forest habitats that have registered changes following the impact of the exercise of this category of plants were presented, and in the present study, the practical Natura 2000 habitats that were affected to a lesser or greater extent by invasive and potential plant species are presented. invasive. These are: 6120 Xeric sand calcareous grasslands, 6240* Sub-pannonic steppic grasslands, 6260* Pannonic sand steppes and 62C0* Ponto-Sarmatic steppes. A summary analysis shows that all analyzed habitats are of community interest, which leads us to say that taking measures to reduce the impact exerted by this category of plants is strictly necessary. The most affected surfaces are those on the periphery of protected areas, where numerous spontaneous species are affected by various phytopathogenic agents and where the zoo-anthropogenic factor makes its presence felt.*

Key words: *grassland habitat, invasive plants, Natura 2000, Oltenia, Romania.*

INTRODUCTION

As compared to the protection of a species, the research and the conservation of natural habitats represent a priority (Noss, 1996; Cowling et al., 2004; Nicholson et al., 2009; Berg et al., 2014; Keith et al., 2015; Timis-Gansac et al., 2022; Georgescu et al., 2023; Georgescu & Luchian, 2023).

Numerous natural habitats that are included in Natura 2000 network of protected natural areas are located within Oltenia region. Some of them refer to grasslands, i.e. to these specific ecosystems, in which all phenomena are continuously subject to the influence of ecological factors.

When referring to Annex I of the Habitats Directive (92/43/EEC), it can be stated that, based on their distribution area, the grasslands under study belong to the following main categories: "Natural grasslands", distinguished by code 61 (for the habitat 6120* Xeric sand calcareous grasslands) and " Semi-natural dry grasslands and scrubland facies" (for habitats: 6240* Sub-pannonic steppic grasslands, 6260* Pannonic sand steppes and 62C0* Ponto-Sarmatic steppes).

The distribution of grasslands on the Romanian territory is uneven because of the great orographic and geobotanical variability (in reference to the mountain, subalpine and alpine regions) and because of the human influence exerted mainly in the plain and hilly regions (Pușcaru-Soroceanu et al., 1963). Out of more than 4 million ha of grasslands and hayfields, approximately half are formations of mesoxerophilous grasslands from hills and plains (about 29% of the total area) and xerophilous steppe grasslands from plains (about 13.5%) (Pușcaru-Soroceanu et al., 1963).

Over time, the natural grasslands within Oltenia have been important for animal feeding, either through grazing or haying (Pavel, 1973). At the beginning of the 20th century, the breeding of domestic animals was not conducted on such a large scale as to induce significant damage to grasslands and to require improvement works. Subsequently and gradually, their irrational use, combined with the lack of proper measures, led to a decrease in the phyto-diversity and productivity of these places.

Scientific studies that mainly aimed at researching the grassland flora and vegetation within Oltenia were carried out over time (Buia & Popescu, 1952; Buia & Păun, 1960; Buia et al., 1961; Ciurchea, 1965; 1971; Cîrțu, 1971; 1976; Păun, 1966; Păun & Popescu, 1975; Popescu, 1979; 1981; 1992; 1996; Rădușoiu, 2005; 2006; Costache, 2011; Niculescu, 2015; 2020). Their main objective was to understand the floristic and vegetation diversity of these ecosystems. With the rapid development of invasive species, some areas occupied by grasslands have undergone obvious changes, this being especially true for those located in the plains and piedmont hills (Acatrinei et al., 2024).

The increases in climate changes extreme events might cause higher yield variability, lower harvestable yields, reductions/extensions in land use in some areas, introduction of new crop species and also changes in crop host and pathogens and pest's interaction, which will drive emergence of infectious diseases in both agricultural and non-managed ecosystems through multiple pathways (Durău et al., 2021; Paraschivu et al., 2022; Paraschivu et al., 2023; Sărățeanu et al., 2023; Velea et al., 2021).

MATERIALS AND METHODS

Oltenia is located in the south-western part of Romania and it is characterized by a great variability related to climatic conditions, relief, and soil types. This variability determines the installation of a significantly diverse flora and vegetation.

From a geographical point of view, Oltenia includes the following regions: the Oltenia Plain, the Getic Piedmont, the Subcarpathian Depression, as well as the mountain, subalpine and alpine regions of the Southern Carpathians - the Parâng and Căpățânii Mountains (Figure 1).

The habitats under study are located within the Oltenia Plain and the Getic Piedmont, i.e. within those regions where zoo-anthropogenic activities are present on a large scale. It should be noted that the analyzed areas are either in the precincts of protected natural areas, or in other areas where research was carried out only from the floristic or vegetation point of view.

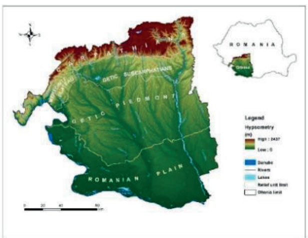

Figure 1. Map of Oltenia

For larger areas, the aerial photography technology used by means of drones is recommended, as it can provide valuable images concerning the areas occupied by the different natural habitats (Călina et al., 2020).

The interpretation of the habitats under study started from the information present in the work entitled "Manual de interpretare a habitatelor Natura 2000 din România (Romanian Manual for Interpretation of EU Habitats)" (Gafta & Mountford, 2008).

The identification of invasive species in certain areas and their rapid proliferation in a short time span has led us to pay close attention to these areas.

For each invasive or potentially invasive species, the population typology was specified by using a scale from 1-5 (1 - solitary organisms; 2 - rare populations, on surfaces < 10 m^2; 3 - rare populations, on surfaces >10 m^2; 4 - dense populations, on surfaces <10 m^2; 5 - dense populations, on surfaces >10 m^2). Furthermore, the introduction mode was also specified in each case.

The scientific names for the species mentioned in the paper are in accordance with POWO (2023).

RESULTS AND DISCUSSIONS

The study conducted for the present paper focuses on priority habitats.

The data gathered over time have highlighted some changes on certain areas occupied by these habitats and this is due to the presence of some invasive and potentially invasive species (Table 1). The comparative analysis of the data collected from surfaces where invasive species were identified and of those originating on surfaces where they are absent, highlights the decline of phytodiversity in the affected habitats.

In contrast to the forest habitats, in the case of the areas occupied by the grasslands included in the analyzed habitats, there can be noticed the presence of invasive and potentially invasive allogeneic species in greater numbers. Nevertheless, a favorable aspect is represented by the fact that few of them have dense populations (e.g. *Ambrosia artemisiifolia* L., *Ailanthus altissima* (Mill.) Swingle), the rest being under the form of solitary individuals or having rare populations (Table 1).

Table 1. Invasive and potentially invasive plants from the researched habitats

Scientific name	Population typology				Introduction mode
	Habitat 6120*	Habitat 6240*	Habitat 6260*	Habitat 62C0*	
Ambrosia artemisiifolia L.	4	2	1	2	accidental
Erigeron annuus (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz	2	2	3	1	accidental
Ailanthus altissima (Mill.) Swingle	1	3	4	2	accidental
Erigeron canadensis L.	3	1	2	1	accidental
Xanthium orientale L. subsp. *italicum* (Moretti) Greuter	2	1	2	1	accidental
Gleditsia triacanthos L.	1	2	2	1	accidental
Oxalis dillenii Jacq.	3	1	1	1	accidental
Robinia pseudoacacia L.	3	1	1	2	accidental
Lycium barbarum L.	1	2	1	1	ornamental
Acer negundo L.	1	1	1	1	accidental
Asclepias syriaca L.	1	-	2	1	accidental
Veronica persica Poir.	1	1	-	-	accidental
Abutilon theophrasti Medik.	1	1	1	-	accidental
Morus alba L.	1	1	1	2	accidental
Amorpha fruticosa L.	-	-	1	2	accidental
Amaranthus powellii S.Watson	1	1	-	1	accidental
Datura stramonium L.	1	-	-	-	accidental
Sorghum halepense (L.) Pers.	1	1	1	-	accidental
Elaeagnus angustifolia L.	-	1	-	2	accidental
Helianthus tuberosus L.	-	1	-	1	accidental
Phytolacca americana L.	1	1	1	2	accidental
Bassia scoparia (L.) A. J. Scott	2	1	-	1	accidental
Euphorbia maculata L.	1	1	2	-	accidental
Oxalis corniculata L.	1	1	1	1	accidental
Panicum capillare L.	1	1	1	-	accidental

Population typology: 1- solitary individuals; 2 - rare populations, on surfaces < 10 m^2; 3- rare populations, on surfaces > 10 m^2; 4-dense populations, on surfaces < 10 m^2; 5- dense populations, on surfaces > 10 m^2.

Understanding the rapid development of certain invasive and potentially invasive plant species, we believe that urgent measures are needed in order to reduce the impact exerted by these plants on grassland habitats, especially on those of community importance.

Habitat 6120* Xeric sand calcareous grasslands, is present on limited areas in Oltenia. It brings together fallow grasslands, which prefer dry places and soils rich in calcium.

In Oltenia, it is known both from Natura 2000 protected areas (e.g. "Coridorul Jiului"), as well as from other areas in the Danube floodplain (e.g. Pisculeț settlement - Dolj county or Izvoarele and Balta Verde settlements from Mehedinți county) (Sanda et al., 2001). The vegetation of this habitat, classified as *Molluginetum cervianae* Borza, 1963, is mentioned from the Jiu river floodplain, near the Tâmburești settlement. Because of the zoo-anthropogenic factor and the appearance of invasive species, *Hypertelis cerviana* (L.) Thulin is present only in isolated specimens (Figure 2), the surfaces where it forms compact phytocenoses being dominated by *Plantago indica* L. (Figure 3) and evolving towards the vegetation characteristic for the habitat 2130* [Fixed coastal dunes with herbaceous vegetation (grey dunes)].

Figure 2. Surfaces where *Hypertelis cerviana* is present

Figure 3. The current state of some areas where habitat 6120* was present - vernal aspect

6240* Sub-pannonic steppic grasslands are known from the Oltenia hills and from the Mehedinți Plateau. Within the "Coridorul Jiului" protected area, it is to be found in the northern part of the site, on dry, sunny areas. The presence of invasive species in the areas occupied by this habitat in Oltenia is explained by the affinity between humans and these places. A significant part of these areas represent former agricultural lands that have not been cultivated for many years, the current vegetation succeeding plant communities dominated by annual species.
This habitat is present on limited areas within the Danube and the Jiu floodplains. The phytodiversity decline in the grasslands occupied by the vegetation of this habitat is due to overgrazing by sheep. Thus, these areas experience the proliferation of ruderal species, whether native, not consumed by sheep, or invasive and potentially invasive alien species (e.g. *Ambrosia artemisiifolia* L., *Ailanthus altissima* (Mill.) Swingle (Figure 4), *Erigeron annuus* (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz).

Figure 4. *Ailanthus altissima* on surfaces occupied by habitat 6240* in the Danube floodplain

6260* Pannonic sand steppes represents a habitat with a greater spread on the continental sands located in the main river floodplains in Oltenia. It is found on flat lands with sandy dunes. The floristic composition of this habitat is characterized by the presence of numerous annual species. As a correspondent to the habitats in Romania, these surfaces are included in R6405 Ponto-Pannonian grasslands on unfixed continental dunes with *Bromus tectorum* (Doniță et al., 2005). It has a high conservative value (Popescu, 2005).

Although this habitat naturally recovers very easily, in some areas of the Jiu and the Danube floodplains, it also displays obvious changes in the floristic composition and structure. This fact is triggered by the practice of irrational grazing or by the simple transformation of these areas into agricultural land.
Among the invasive and potentially invasive alien species that exert a greater impact on the areas occupied by this habitat in Oltenia, there are to be noted: *Erigeron annuus* (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz, *Ailanthus altissima* (Mill.) Swingle, *Erigeron canadensis* L., *Xanthium orientale* L. subsp. *italicum* (Moretti) Greuter, *Gleditsia triacanthos* L., *Asclepias syriaca* L. (Figure 5).

Figure 5. Surfaces within the habitat 6260* from Oltenia, which are affected by invasive alien species (*Ailanthus altissima* and *Erigeron annuus* subsp. *strigosus*)

The habitat 62C0* Ponto-Sarmatic steppes is found in several areas of Oltenia. It is also mentioned from the precincts of certain Natura 2000 protected areas (e.g. Silvostepa Olteniei).
From the coverage viewpoint, this habitat has a good representation, occupying the second place after the forests of turkey oak and Hungarian oak with *Festuca heterophylla* in the weedy layer.
On certain areas, the conservation value of this habitat is very high due to the presence of vascular species of sozological importance: *Ziziphora capitata* L., *Sternbergia colchiciflora* Waldst. et Kit., *Salvia aethiopis* L., *Crucianella angustifolia* L., *Convolvulus cantabricus*, *Lathyrus sphaericus* M. Bieb., etc. The surfaces occupied by the vegetation of this habitat are used in most cases as pastures and they are found on flat, fertile, dry lands, either on chernozems or on alluvial soils (Sanda et al., 2008) (Figure 6).

Figure 6. Physiognomy of the areas occupied by the 62C0* habitat within Oltenia

In some locations with Oltenia, the vegetation of this habitat is affected either by invasive native species (e.g. *Lepidium draba* L.), or by alien species: *Ambrosia artemisiifolia* L., *Ailanthus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L., *Morus alba* L., *Amorpha fruticosa* L., *Elaeagnus angustifolia* L., *Phytolacca americana* L., as well as by other taxa that are present as solitary individuals.

CONCLUSIONS

The comparative analysis of the four habitats presented above, by taking into account the presence of invasive and potentially invasive allogeneic species, highlights the fact that this category of plants has poor populations in the investigated areas. There are few cases in which these plants have a greater spread, with rich populations (e.g. *Ambrosia artemisiifolia* in habitat 6120*). However, if we take into account the fact that the analyzed habitats are of community importance and the invasive alien species in these habitats are in a fairly large percentage, compared to those identified in Romania (between 15-20% of the total), we can say that the need to protect these habitats is acute.

Table 1. Invasive and potentially invasive plants from the researched habitats

Scientific name	Population typology				Introduction mode
	Habitat 6120*	Habitat 6240*	Habitat 6260*	Habitat 62C0*	
Ambrosia artemisiifolia L.	4	2	1	2	accidental
Erigeron annuus (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz	2	2	3	1	accidental
Ailanthus altissima (Mill.) Swingle	1	3	4	2	accidental
Erigeron canadensis L.	3	1	2	1	accidental
Xanthium orientale L. subsp. *italicum* (Moretti) Greuter	2	1	2	1	accidental
Gleditsia triacanthos L.	1	2	2	1	accidental
Oxalis dillenii Jacq.	3	1	1	1	accidental
Robinia pseudoacacia L.	3	1	1	2	accidental
Lycium barbarum L.	1	2	1	1	ornamental
Acer negundo L.	1	1	1	1	accidental
Asclepias syriaca L.	1	-	2	1	accidental
Veronica persica Poir.	1	1	-	-	accidental
Abutilon theophrasti Medik.	1	1	1	-	accidental
Morus alba L.	1	1	1	2	accidental
Amorpha fruticosa L.	-	-	1	2	accidental
Amaranthus powellii S.Watson	1	1	-	1	accidental
Datura stramonium L.	1	-	-	-	accidental
Sorghum halepense (L.) Pers.	1	1	1	-	accidental
Elaeagnus angustifolia L.	-	1	-	2	accidental
Helianthus tuberosus L.	-	1	-	1	accidental
Phytolacca americana L.	1	1	1	2	accidental
Bassia scoparia (L.) A. J. Scott	2	1	-	1	accidental
Euphorbia maculata L.	1	1	2	-	accidental
Oxalis corniculata L.	1	1	1	1	accidental
Panicum capillare L.	1	1	1	-	accidental

Population typology: 1- solitary individuals; 2 - rare populations, on surfaces < 10 m^2; 3- rare populations, on surfaces > 10 m^2; 4-dense populations, on surfaces < 10 m^2; 5- dense populations, on surfaces > 10 m^2.

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THE IMPACT OF THE SPECIES *ELODEEA NUTTALLII* ON NATURAL AQUATIC HABITATS IN OLTENIA, ROMANIA

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Abstract

Elodea nuttallii (Planch.) H. St John is a native taxon from North America, which is increasingly widespread in Oltenia, in recent years. It has been known in the flora of Romania since 1993. At the European level, it is on the list of invasive species of interest for the EU. In Oltenia, it prefers aquatic habitats with stagnant or smoothly flowing water. However, it grows in very good conditions and in cloudy waters, where very little light penetrates, a fact that gave this species an advantage compared to the plants it coexists with, greatly reducing their number. Following the research carried out by us, we noticed that in some aquatic habitats in Oltenia this plant registers low dominance abundance indices, but in others it builds up monodominant plant communities or with very few other vascular species, which causes biodiversity to decrease in these places very much. Among the aquatic habitats affected by the presence of the species Elodea nuttallii we mention: 3150 Natural eutrophic lakes with Magnopotamion or Hydrocharition-type vegetation and 3160 Natural dystrophic lakes and ponds.

***Key words**: aquatic habitats, Elodea nuttallii, impact, Oltenia, Romania.*

INTRODUCTION

Although floristic and vegetation studies have been conducted since the end of the 19th century in this part of Romania, it cannot be stated that this research work on vascular flora and natural and semi-natural vegetation is completed at present. A strong evidence is provided by the research carried out by the botanists of the Craiova University Centre until 2006, regarding the aquatic flora and vegetation (Buia & Popescu, 1952; Păun, 1967; Păun & Popescu, 1969; Cârțu, 1972; Cârțu, 1976). To this date, no publication includes a study of the species analysed by us. After 2001, it appears in several scientific works, but only in the floristic list or in the composition of certain phytocoenoses belonging to other associations (Popescu et al., 2001; Costache, 2005; Costache, 2011; Răduțoiu, 2011).

Before discussing how climate change affects plant constrainers, it is necessary to take into account how the world's climate may affect the dynamics and population structure of plants, the micro-evolutionary processes and structure of plant communities, as well as crop yield and quality (Dima et al., 2023; Sălceanu et al., 2023; Sărățeanu et al., 2023). Furthermore, there will be crop relocation, diseases associated with changes in the atmospheric composition and global climate with economic consequences from crop loss and changes in host-pathogen relationship (Paraschivu et al., 2022; Paraschivu et al., 2023). The climate changes occurred during the last 25 years have triggered obvious changes in the floristic composition of the phytocoenoses of certain plant associations (Niculescu, 2016; 2023), favouring the spread of some adventitious species with invasive potential (e.g. *Elodea nuttallii*).

The analysis of the different natural and semi-natural habitats in Oltenia underlined the fact that the aquatic habitats are the ones that have registered the most noticeable changes in the floristic composition, in the framework of climate changes during recent years.

The allogeneic alien species have gained ground over native species (Lambdon et al., 2008), becoming invasive in certain habitats (Georgescu & Luchian, 2023; Costache et al., 2021). In these places, they produce major changes both in terms of phytoplankton and zooplankton, inducing significant damage in the economy of these areas (Paini et al., 2016).

In recent years, it has been noticed that *Elodeea nuttallii* (Planch.) H. St. John also settles in artificial, man-made aquatic habitats (Chytrý et al., 2009; Wang et al., 2019).

The changes in aquatic habitats are explained, on the one hand, by the actions of humans and domestic animals (Thiébaut, 2007; Fleming & Dibble, 2015), which enter these habitats where they leave their visible mark during the warm periods of the year; on the other hand, they are triggered by water fluctuations, which are increasingly frequent in recent years.

Elodea nuttallii (Planch.) H. St. John is an adventive species of EU interest, originating in North America (Figure 1).

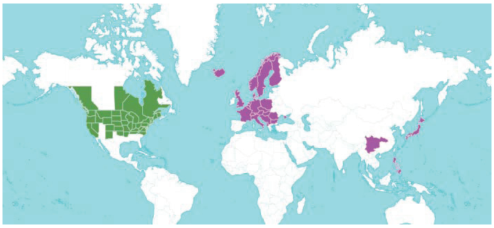

Figure 1. Distribution of the species *Elodea nuttallii* (POWO, 2023)

The presence of this species in the European flora dates back to 1939, when it was mentioned from Belgium. From Kempen and the Schelde river valley it is cited as a rare species (Van Rompaey & Delvosalle, 1979). Its presence in the flora of Romania dates back to 1997, from the Danube Delta (Ciocârlan et al., 1997). Over time, this species has been found to gradually replace *Elodea canadensis*, as the stems and branches of *Elodea nuttallii* grow much faster as compared to cu *E. canadensis*; moreover, *Elodea nuttallii* is a species with higher tolerance to low light (Simpson, 1990; Greulich & Trémolières, 2006).

Elodea nuttallii is a plant that tolerates the oligotrophic, as well as the eutrophic sites and even the polluted ones (Samecka-Cymerman & Kempers, 2003; Grinberga & Priede, 2010; Dodkins et al., 2012). Moreover, in recent years, it has been found that due to the increase in temperatures in southern Romania, this plant remains green even during the winter, favouring a faster entry into vegetation in the spring of the following year. This fact has serious consequences for the waters in which the species is present, because it occupies the entire water mass in a relatively short time (Kunii, 1984; Zehnsdorf et al., 2015). The specialized literature states that *Elodea nuttallii* grows well in dirtier, polluted waters, unlike *E. canadensis*, which is found in clear, clean and cold waters (Ciocârlan et al., 1997).

The main purpose of the present study is to show the impact of the invasion exerted by *Elodea nuttallii* within the plant communities where it settles in Oltenia. The authors accomplished that aim through the comparative analysis of unaffected habitats and of those where this species occupies the entire water table.

MATERIALS AND METHODS

Information on the chorology of this species in Oltenia was obtained from the specialised literature, various sources mentioning the species in the floristic inventory of the studied areas (Popescu et al., 2001; Costache, 2005; 2011; Răduțoiu, 2011). Valuable information was also obtained from the main herbaria of the country (Bucharest, Iași, Cluj-Napoca, and Craiova), as well as from the field trips conducted by the author in different areas of Oltenia.

By comparing the existing data in the specialized literature with the information collected from the field, the author was able to assess the impact triggered by the development of the species *Elodea nuttallii* on the aquatic habitats in which it is present.

The scientific plant names used in this material are in accordance with POWO (2023). The acronyms for the consulted herbariums are in accordance with the Index Herbariorum (Thiers 2022+): Iași ("Alexandru Ioan Cuza" University Herbarium - I), Cluj-Napoca ("Babeş-Bolyai" University Herbarium in Cluj-Napoca - CL), Bucharest (Herbarium of the Institute of Biology of the Romanian Academy - BUCA; Herbarium of the "D. Brândza" Botanical Garden in Bucharest - BUC), Craiova (Herbarium of the University of Craiova - CRA).

The distribution maps were achieved by using the RoBioAtlas program (2023), which displays the species distribution in correlation with the average annual precipitation and temperatures. On these maps, the points where this species is invasive are marked in red, while the green marks identify the places where it is present in the floristic composition of other plant associations, where it has low Abundance-Dominance indices.

RESULTS AND DISCUSSIONS

The areas where *Elodea nuttallii* is characterised by a significant development, inducing obvious changes on these types of habitats in Oltenia, have been identified in several places in the counties of Gorj (e.g. Rovinari), Mehedinți (Lunca Banului), Olt (Gostavățu), and Dolj (Sadova, Bădoși, Ciupercenii Noi, Desa, Piscu Sadovei, and Ciupercenii Vechi). Furthermore, this taxon is present in several other places in Oltenia, without showing invasive potential. The comparative analysis involving the structure of the phytocoenoses of the association dominated by this plant attests to the existence of certain differences in terms of floristic composition and water quality.

The association edified by *Elodea nuttallii* was described for the first time in the field literature under the name of *Ceratophyllo demersi – Elodeetum nuttallii* Ciocârlan, Sârbu, Ștefan, Marian 1997 and it was included in the following coenotaxonomic system: *Potamogetonetea pectinati* Tx. et Prsg. 1942, *Potamogetonetalia pectinati* W. Koch 1926, *Potamogetonion lucentis* Rivas Martinez 1973 (Ciocârlan et al., 1997).

In the samplings presented by the authors of the work, from the Danube Delta, the presence of 14 taxa is noticed. The nomenclatural type of the association is represented by sampling number 3 (Ciocârlan et al., 1997).

Other phytocoenoses of this association, in which the presence of 18 taxa can be observed, are described from the Danube Delta Biosphere Reserve, more precisely from the area of Dranov and Belciug lakes (Ștefan et al., 2006). Moreover, 10 species from the floristic composition of this association are mentioned from Mărașu locality (Brăila county) (Sanda et al., 2005).

It is important to mention that the species *Elodea nuttallii* is known from few aquatic communities at the European level (Rodwell, 2000; Weekes et al., 2018).

Although the species analysed by us is present both in habitat 3150 - Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation, as well as in habitat 3260 - Watercourses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation, the invasive potential is manifested only in the habitat 3260. This is due to the fact that the water of the latter habitat has a slow and permanent flow, also being shallower than that of habitat 3150; this characteristic induces the near absence of the floating vegetation, thus favouring the development of submerged plants (Figure 2).

Figure 2. *Ceratophyllo demersi - Elodeetum nuttallii* (Ciocârlan et al., 1997) from the Jiu meadow, near Sadova settlement (original)

The analysis of the association table (Table 1) underlines the fact that the surveys carried out in different areas of Oltenia point to the very small number of species (3-4) with which it coexists. This is also reflected in the ichthyofauna of these areas, the number of fish species being greatly reduced. The conducted studies pointed out to the fact that those that still survive are small in size (Figure 3). In the end, there will be major disturbances at the level of the entire food chain.

Figure 3. Fish species from habitats where *Elodea nuttallii* este is invasive - Rovinari (Gorj county)

Chorology: In order to represent the distribution of the *Elodea nuttallii* species, we used the RoBioAtlas program, which enables correlations with average annual temperatures (Figure 4) and average annual precipitation quantities (Figure. 5).

Table 1. *Ceratophyllo demersi - Elodeetum nuttallii* Ciocârlan, Sârbu, Ştefan, Marian 1997

Proveniența releveurilor	1	2	3	4	5	6	7	8	9
Acoperirea vegetației (%))	90	85	90	90	85	90	90	85	90
Adâncimea apei (cm)	90	120	150	125	95	80	95	65	75
Suprafața (m^2)	5	4	4	25	30	12	10	4	4
Expoziția	-	-	-	-	-				
Elodea nuttallii	5	4	4	5	4	4	5	4	5
Ceratophyllum demersum	-	+	1	+	+	1	-	+	+
Potamion et Potametalia									
Potamogeton pectinatus	+	1	-	-	-	-	-	-	-
Potamogeton pusillus	-	-	+	-	-	+	-	+	
Potamogeton crispus	-	+	-	+	+	-	-	+	+
Potamogeton nodosus	-	-	+	+	+	-	+	+	+
Myriophyllum verticillatum	+	-	-	-	-	-	-	-	+
Ranunculus trichophyllus	+	+	-	-	+	+	-	+	-
Hydrocharition									
Hydrocharis morsus-ranae	-	-	-	-	-	+	-	+	-
Salvinia natans	-	+	-	-	-	+	-	+	+
Lemnion									
Lemna minor	+	+	+	+	+	-	-	+	+
Lemna trisulca	-	-	+	-	-	+	-	-	-
Azolla filiculoides	-	+	-	-	-	-	-	-	-
Nymphaeion									
Trapa natans	-	+	-	-	-	-	+	+	-
Nymphoides peltata	-	-	-	-	-	-	+	-	-
Phragimtetea australis									
Typha angustifolia	-	-	-	+	-	-	-	-	+
Sparganium erectum	-	-	-	+	-	-	-	+	+
Alisma plantago-aquatica	-	-	-	-	-	-	+	-	-

The provenance of the reliefs: 1. Lunca Banului (Mehedinți County) (13.06.2012); 2. Canal in the meadow of Olt next to the town of Gostavătu (Olt County) (01.06.2013); 3. Sadova - in the Jiului meadow (Dolj County) (12.10.2014); 4. The city of Bădoși, along a canal in the Jiului meadow (Dolj County) (30.05.2015); 5. Desa, along a channel near the Pietrile pond (Dolj County) (09.06.2015); 6. Ciupercenii Vechi - in Ciuperceni Pond (Dolj County) (09.06.2015); 7. The outskirts of Rovinari (județul Gorj) (26.08.2023); 8. Ciupercenii Noi (Dolj County) (16.07.2015); 9. Piscu Sadovei in Zăcătoarea Pond (14.09.2019).

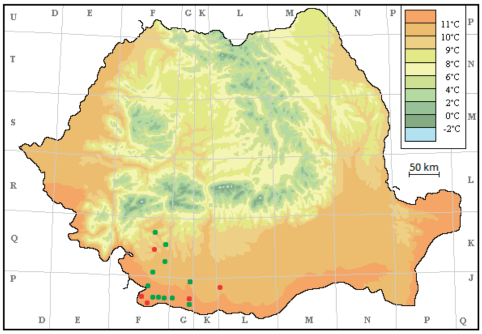

Figure 4. Distribution of the species *Elodea nuttallii* in Oltenia, in correlation with the average annual temperatures

The species analysed in the present paper was identified in the following settlements: Lunca Banului, in Debarcader Lake (Mehedinți County), Gostavățu (Olt County), Rovinari, Izvoarele, Ceplea and Bâlteni (Gorj County), as well as Bădoși - along a canal, Ciupercenii Noi, Desa - along a canal near the Pietrile pond, Sadova, Piscu Sadovei - in the Zăcătoarea pond, Ostroveni, on the outskirts of Calafat, Ciupercenii Vechi - Ciuperceni pond, Rast, Bistreţ, Ghidici, Dunăreni, Grindeni, Hunia, Salcia, and Lişteava (Dolj).

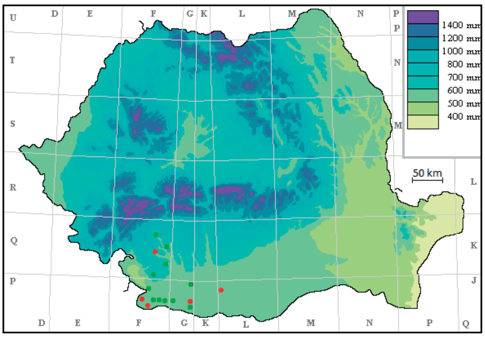

Figure 5. Distribution of the species *Elodea nuttallii* in Oltenia, in correlation with the average annual precipitation quantities

In addition, the presence of the species *Elodea nuttallii* in other areas from southern Oltenia enables us to state that the invasive potential of this plant will also become obvious in these places in the near future, the taxon being favoured by the zoo-anthropogenic factor (Figure 6) and by the climate changes of recent years.

Figure 6. The influence of the zoo-anthropogenic factor on the aquatic vegetation in southern Oltenia

CONCLUSIONS

Following the research carried out, we can state that the increasing dispersion of *Elodea nuttallii* species can be attributed to water pollution. The main factor that triggers changes in the floristic composition of aquatic habitats is the zoo-anthropogenic one. On the other hand, the decreasing water regime during recent years creates new favourable habitats for *Elodea nuttallii*.

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MORPHOLOGICAL, ANATOMICAL AND PHYSIOLOGICAL LEAF TRAITS OF PISTACHIO (*PISTACIA VERA* L.) GROWN IN BUCHAREST AREA (ROMANIA)

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Abstract

Pistachio (Pistacia vera L.) is a very important crop species due to its nutrient-rich nuts, as well as its special ability to adapt to climate changes. Micromorphological, anatomical and physiological characteristics have been performed in the leaf taken from the male tree, grown in the Bucharest area (Romania). The samples, represented by mature leaves, were collected in June-July. Leaves are simple, but also trifoliate, with ovate leaflets, with entire margins, glossy. The leaf micromorphology was performed by scanning electron microscopy. For anatomical observations, epidermis was collected from both sides of the leaf, transverse sections were carried out in the leaf lamina and petiole, then observations have been done using light microscopy. Stomata are of anomocytic type, located on both sides of the leaf. The secretory ducts have been identified in the phloem of the vascular bundle of the petiole and in the middle vein of the leaf lamina. The leaf mesophyll is equifacial, having the palisade tissue under both epidermises, and the spongy tissue in situated in the middle area. Physiological indicators can be useful for the characterization of the pistachio adaptation mechanisms in the resilience integrated framework.

Key words*: Pistacia vera, anatomy, morphology, physiology.*

INTRODUCTION

The genus *Pistacia* is characterized by a special ecological and phenotypic plasticity (El Zerey-Belaskri, 2019), as well as considerable genetic diversity (Hakimnejad et al., 2019). Although it shows increased tolerance to the stress factors action, arid and semi-arid regions where it usually grows are known for exhibiting extreme stress conditions, especially regarding drought and salinity levels (Behboudian et al., 1986; Mehdi et al., 2011; Ben Hamed et al., 2023), which are higher than usual (Esmaeilpour et al., 2016b). Low temperatures can also be added, which in certain areas may impair the species performance (Ravari et al., 2023), as is the case in Romania. Thanks to the ecological plasticity, both at the individual and/or the population level, it is possible to adapt to the temporal and/or spatial variations of the different environmental limiting factors by developing some regulatory mechanisms which determine morphological and physiological changes, as recently demonstrated in *Pistacia lenticus* (Doghbage et al., 2023). The attention given to it is also supported by a recent exhaustive review carried out by Nezami and Gallego (2023), which deserves to be studied carefully to create an ensemble image of this genus, from its origins to the present, and more than that on the perspective concerns on this topic.

Pistachio (*Pistacia vera* L.) is the most economically important species in the genus *Pistacia* (FAOSTAT, 2023; cited by Miri et al., 2023), recognized as such due to its unique nutrient composition, as well as its special ability to adapt to climate changes (Bailey et al., 2020).

Consequently, the knowledge of behavior in different conditions, the possibilities of adaptation to new environmental areas, as well as the increase of resilience to environmental challenges must be in the attention of researchers and farmers in the areas where this species is usually cultivated and why not also where its growth would be possible. As mentioned by Hakimnejad et al. (2019) after studying 42 genotypes belonging to different pistachio species (including *P. vera*), significant positive correlations registered between morphological, eco-physiological and photosynthetic indicators can represent a starting point for improvement of the breeding programs. Notably, the 'Siirt'cultivar belonging to *P. vera* (included in the first group by cluster analysis) can be used in breeding programs as parents, to increase the degree of tolerance of rootstocks and cultivars to environmental conditions.

P. vera being a dioecious species and as it is well known, for such species one of the adaptation strategies related to sex (males and females) is represented by the structural and functional characteristics of the leaves (Korgiopoulou et al., 2019). The research team conducted a comparative study on 50-year-old trees (females with low fruit load and males), both in sun and shade conditions. For the male trees, the leaves were smaller and total conducting petiole area (TCA) was significantly smaller, while the stomatal density, the water use efficiency and the content of phenolic substances had higher values. In the case of the female trees, the leaves had a larger surface, with a greater thickness, higher values of the leaf mass/leaf area, as well as higher values of TCA and, respectively, of maximum photosynthetic capacity per area ($A_{max,a}$). It seems that, female trees stronger invest in xylem efficiency and carbon gain, while male trees invest more in defense. The phenotypic plasticity of the sexes in response to sun and shade conditions was approximately the same, with the remark that each sex expresses different optimization strategies.

To our knowledge, in Romania, no studies have been done from this point of view. Therefore, our results hope to bring some data regarding the morphological, micromorphological and physiological traits of the male specimen over time, including preparation for the winter season, in the case of the temperate continental climate specific to our country.

MATERIALS AND METHODS

Biological material

The study has been performed using the collected leaves from the male tree of *Pistacia vera* L., grown as ornamental species in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania (USAMV Bucharest).

The specimen has been brought 35 years ago from Grece to be acclimatized and studied in the Bucharest area (Romania).

The Bucharest city is located at 44º24′49″ North latitude and 26º5′48″, East longitude, 90 m altitude and temperate-continental climate, with 585 mm/year rainfall and 10.86°C the average of the temperature.

On the active growth season, in June and July months, the leaves of pistachio have been collected from the branches located at the height of 1.5 m within South-West exposure and morphological, micromorphological and anatomical observations were made on the collected leaves.

Micromorphological and anatomical assessment

The micromorphology of the petiole and leaf lamina has been assessed with the scanning electron microscope (SEM) belonging to the laboratory of microscopy and plant anatomy in the USAMV Bucharest research center.

For anatomical analysis have been performed the transversal sections in the leaf petiole and in the leaf lamina and there were clarified with Chloral-hydrate coloured with the Carmine-Alaunate and Iodine Green according to the classic method (Andrei et al., 1975).

Both epidermises of the leaves were collected and used for counting the number of stomata per square millimeter and for measuring the size of stomata (length, width). In the transversal sections of the petiole and leaf lamina has been measured the size of the specific tissues.

The remarks, images and the measurement of the anatomical structures were made with the optical microscope Leica DM1000 LED, video camera Leica DFC295 belonging to the Laboratory of microscopy and plant anatomy of the same research center.

The photos have been take using the 4X and 20X objectives and the measurements of the tissues were made at the objective of 20X.

For counting of the stomata, the objective 40X has been used. The photos and measurements of the stomata were made at the objective 20X.

Determined physiological indicators

Gas exchange measurements: net photosynthesis (Pn) (μmol CO_2 m^{-2} s^{-1}); transpiration rate (Tr) (mmol H_2O m^{-2} s^{-1}); stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1}) and intercellular carbon dioxide concentration (Ci) (μmol CO_2 mol^{-1}) have been determined during October with the help of a portable photosynthesis system (LCPro+ Bio-Sciences), between 10:00 am and 12:00 pm, for ten leaves still visibly green, sun-exposed, as well as yellowed leaves. Then, the following physiological indicators were estimated: water use efficiency (WUE) (Pn/E); quantum yield (QY) (φCO_2) (Pn/PPFD) and Tr/g_s. Total chlorophyll content was estimated by using the chlorophyll meter CCM-300 and results were expressed as mg/dm^2 FW. The study of membrane stability has been carried out by the conductometric method. Thus, electrolytes transport through membranes (MET) ($\mu S\ cm^{-1}g^{-1}$), and total electrolyte content (TE) ($\mu S\ cm^{-1}g^{-1}$) have been determined. Based on them, the synthetic indicator called electrolyte leakage (EL) (%) was calculated. Statistical analysis has been performed by the Student's T-Test.

RESULTS AND DISCUSSION

Leaf morphology and micromorphology

The male tree specimen of *P. vera* from botanical garden is low size (4 m height), with a short trunk and a strongly branched globular crown.

The leaves are alternate, simple and composed of three leaflets, ovate – elliptical, with entire edge, mucronate or obtuse leaf tip, glossy (Figure 1).

Figure 1. *Pistacia vera* L., male specimen from the Botanical Garden, USAMV Bucharest

As results of micromorphological analysis it has been observed that the leaves had non-glandular hairs on the petiole and on both epidermises of leaf lamina, more numerous in the lower epidermis and the stomata were on both epidermises (Figures 2 a, b, c).

Figure 2 a. Micromorphology of the petiole - *Pistacia vera* L. (SEM 800 X)

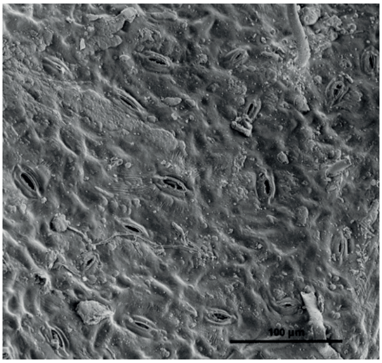

Figure 2 b. Micromorphology of the lamina - analysis of the upper epidermis - *Pistacia vera* L. (SEM 800 X)

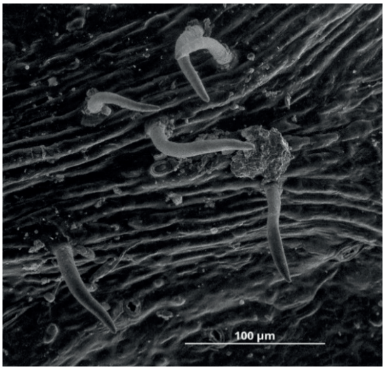

Figure 2 c. Micromorphology of the lamina - analysis of the lower epidermis - *Pistacia vera* L. (SEM 800 X)

Leaf anatomy

The leaf anatomy of the *P. vera*, analyzed male tree is in line with the data from literature (AL-Saghir et al., 2006).

Petiole anatomy

In the transversal section, the petiole is ovoidal with two small lateral prominence in the adaxial zone (Figure 3 a).

From the border to the center of the petiole there are the following tissues: epidermis, collenchyma, parenchyma, vascular bundles and the pith (Figure 3 b).

The epidermis is uniseriate with the medium thickness of 12.4 µm covered by a thick cuticle of 3 µm.

In the epidermis there were observed the stomata and the unicellular non glandular hairs.

The angular collenchyma is 71.2 µm thick.

In the adaxial collenchyma there were identified the chlorenchyma zones.

The parenchyma is formed by ovoidal cells with the thin walls and intracell spaces, having 53.5 µm width average. In the parenchymatic cells there were identified the mineral crystals.

The vascular bundles are collateral-opened, with secondary structure generated by the cambium and bordered by the sclerenchyma and separated by the principal medullar rays.

In the abaxial zone there were identified 6-9 semicircle big vascular bundles and in the adaxial zone were 4-5 horizontal small vascular bundles.

In the phloem, the secretory ducts were identified, having a large lumen with 88 µm.

The thickness of the phloem has been 68 µm and the xylem has been 105 µm.

The pith is star shape with parenchymatic origins and 42 µm size with mineral crystals in the cells.

The micromorphology and the size of the leaf tissues can be significant influenced by the climatic conditions and air pollution with the cars gas emissions (Amara, 2017; Nadjat et al., 2020; Açar, 2023).

Lamina anatomy

The front view of the epidermises presented the polygonal cells with the thin walls (Figures 4 a and b).

The leaf is amphystomatic and the stomata are anomocytic type, like in the speciality literature (AL-Saghir & Porter, 2005).

In the upper epidermis, the average of the stomata density is 105 per mm^2, and 145 per mm^2 in the lower epidermis.

Figure 3 a. Transversal section in the petiole - *Pistacia vera* L. ad.ep - adaxial epidermis; ngh - non glandular hairs; co - collenchyma; pa - parenchyma; sc - sclerenchyma; ph - phloem; xy - xylem; sd - secretory duct; pi - pith; ab.ep - abaxial epidermis (objective 4X)

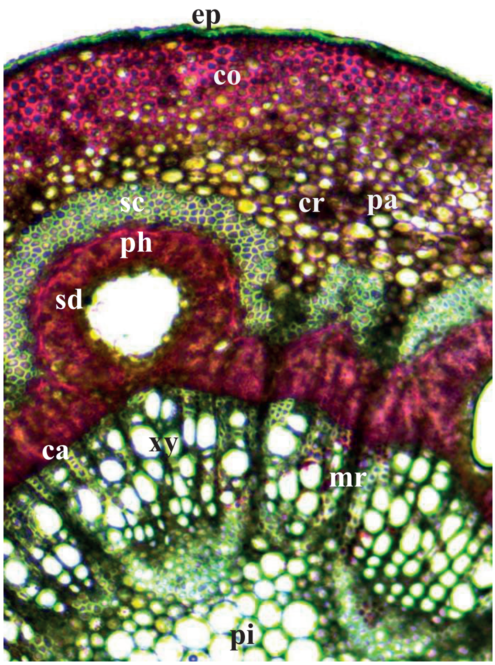

Figure 3b. Transversal section in the petiole (detail) - *Pistacia vera* L.
ep - epidermis; co - collenchyma; cr - crystal
pa - parenchyma; sc - sclerenchyma; ph - phloem;
ca - cambium; xy - xylem; sd - secretory duct;
mr - medullar rays; pi - pith (objective 20 X)

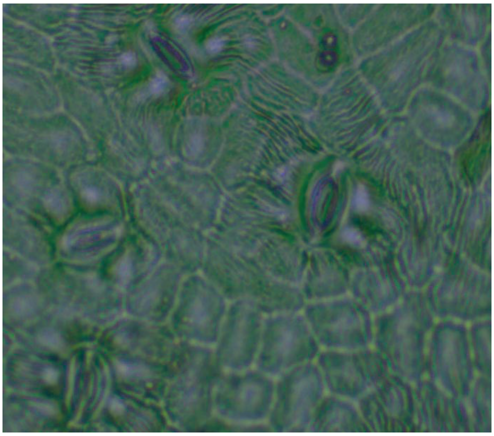

Figure 4 a. Analysis of the upper epidermis of the lamina - *Pistacia vera* L. (objective 20X)

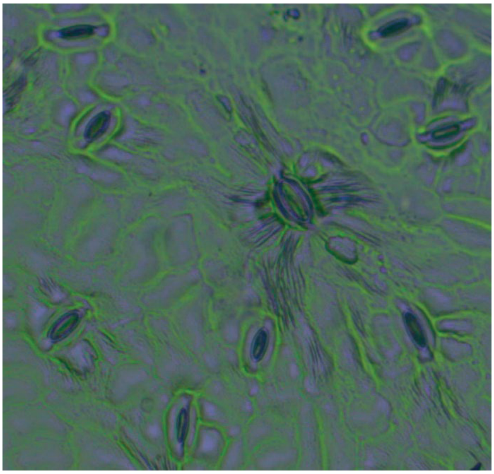

Figure 4 b. Analysis of the lower epidermis of the lamina - *Pistacia vera* L. (objective 20 X)

The average size of the stomata is: 19 µm lenght x 16 ɥm width for upper epidermis and 22 µm lenght x 17 µm width for lower epidermis.
The stomatal density is significantly influenced by the amount of precipitation of the geographical area (AL-Saghir, 2006).
The highest stomatal density and the lowest stomatal length and width were obtained under severe hydric stress (Arzani et al., 2013).
In cross-section through the lamina the epidermises are uniseriate with an average thickness of 16 µm (Figure 5).
The leaf mesophyll is equifacial (bilateral), with palisade tissue under the both epidermises and spongy tissue in the middle.

There are structural differences in the mesophyll of the leaves between different species of *Pistacia* (Leng et al., 2007; Mehdeb et al., 2016).
The palisade tissue below the upper epidermis consists of 2 rows of elongated cells, rich in chloroplasts, with an average thickness of 70 µm.
The palisade tissue located under the lower epidermis consists of 1-2 rows of short cells with an average thickness of 35 µm.

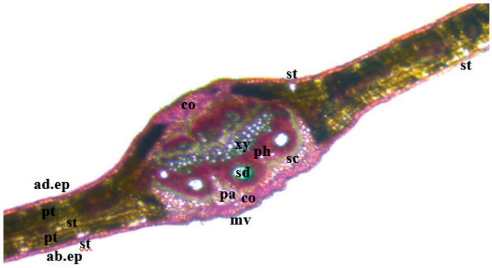

Figure 5. Transversal section in the lamina – *Pistacia vera* L.
ad.ep - adaxial epidermis; pt - palisade tissue; st - spongy tissue; st – stomata; mv - middle vein; co – collenchyma; pa – parenchyma; sc – sclerenchyma; ph – phloem; xy – xylem; sd – secretory duct; ab.ep – abaxial epidermis (obiective 4X)

The spongy tissue is compressed, consisting of 3-4 rows of ovoid cells, with a lower content in chloroplasts and the average of thickness 32 µm.
In the mesophyll there were identified the collateral bundles with the xylem to the adaxial epidermis and phloem to the abaxial epidermis.
In the middle vein of the leaf is ellipsoidal shape with a uniseriate epidermis under which an angular collenchyma has been observed, more developed in the adaxial part and a parenchyma.
In the middle vein were revealed 3-4 collateral vascular bundles, 3 smaller vascular bundles located in the adaxial part and 4 bigger vascular bundles towards the abaxial part.
In the phloem of the vascular bundles from the abaxial part has been identified the secretory ducts, with a diameter of 45 µm.

Leaf physiology

The obtained results can be seen in Table 1. With the exception of Tr/gs (p value = 0.380635), statistically significant differences ($p < 0.05$) were recorded between the still visibly green leaves and the yellowed ones in a proportion of about 75% (yellowed - red) for all the others. Very significant higher values have been obtained for yellowed leaves (Pn, WUE, QY, MET and percentage EL), while lower data can be seen for Tr, g_s, Ci, and TE, as against to the still green leaves.

The explanation could be that during the autumn period, the establishment of senescence of the leaves is a process that takes place gradually and depends largely on the climatic conditions of the respective year, especially those related to the level of day-night temperature variation, the presence or absence of precipitation, the degree of drought etc. For the visibly green leaves, it is possible that the reduced values of Pn (and of the associated indicators), are due to the intensification of the respiration process, the rate of which exceeds the rate of Pn. Regarding the transpiration rate, the low values for leaves in an advanced degree of yellowing may be due to the drastic decrease in leaf turgor (implicitly of the stomata guard cells) and as a result the stomata close. This behavior is also supported by the reduced values of g_s (1.62 times lower values for YL vs. GL).

As for the membrane stability, the significant differences are noted from a statistical point of view for each individual indicator, but it is worth noting that in the case of yellowed leaves, the EL (%) was consistently and significantly higher, almost double compared to green leaves. This behavior is in close relation with the reduction of the degree of selectivity of the membranes together with the installation of senescence of the leaves during the fall. Furthermore, it is interesting that TE content was greatly reduced in the yellowed leaves (about twice), which indicates a redirection of the mineral ions from the leaves to other tissues of the plant, before the leaves fall. It is a normal physiological process of preparing the plants for entering the dormant period and ensuring survival for the next season. An increased value of EL (%) was also recorded in the case of the incident of different stress factors, such as low temperatures in the case of a cold climate. The studies carried out by Ravari et al. (2023) on different pistachio genotypes to select tolerant varieties in such areas, highlighted the specific variability depending on the genotype. Thus, at a temperature of -16 C, for *P. atlantica* and *P. khinjuk* the EL values were the highest, while for *P. terebinthus* and *P. vera* var. Sarakhs there were determined the lowest values for this indicator.

Table 1. Physiological indicators in the leaves of *Pistacia vera* L., in the autumn season

Variable	Green Leaf (GL)	Yellowed Leaf (YL)	the *p* value	Statistical significance (YL versus GL)
(Pn) (net photosynthesis - µmol CO_2 m^{-2} s^{-1})	-0.33 ± 0.10	0.27 ± 0.15	0.006015	**
(Tr) (transpiration rate - mmol H_2O $m^{-2}s^{-1}$	1.29 ± 0.15	0.84 ± 0.07	0.004894	00
(g_s) (stomatal conductance – mol H_2O m^{-2} s^{-1})	0.026 ± 0.005	0.016 ± 0.002	0.017055	0
Intercellular carbon dioxide concentration (Ci) (µmol CO_2 mol^{-1})	418 ± 11.85	390.60 ± 9.81	0.009381	00
Water use efficiency (WUE)(Pn/Tr) (µmol CO_2 m^{-2} s^{-1}/ mmol H_2O $m^{-2}s^{-1}$)	-0.286 ± 0.17	0.2268 ± 0.28	0.006431	**
Quantum yield (QY)(φCO_2) (Pn/PPFD) (µmol CO_2 m^{-2} s^{-1}/ µmol photons $m^{-2}s^{-1}$)	-0.00032 ± 0.000179	0.00018 ± 0.000218	0.004423	**
Tr/g_s (mmol H_2O $m^{-2}s^{-1}$/ mol H_2O m^{-2} s^{-1})	53.82 ± 9.71	55.70 ± 8.63	0.380635	NS
Total estimated chlorophyll content (mg dm^{-2})	6.468 ± 0.125	5.676 ± 0.159	0.005013	00
Electrolytes transport through membranes (MET) (µS cm^{-1} g^{-1})	904.50 ± 3.88	1275.20 ± 16.64	0.010951	**
Total electrolyte content (TE) (µS cm^{-1} g^{-1})	2031.11 ± 7.97	1986.90 ± 4.75	0.023145	0
Electrolyte leakage (EL) (%)	44.53 ± 0.02	64.18 ± 0.68	0.010814	**

Results are shown as mean values ± standar error (SE) (n = 5). The comparison was done by the paired T-Test, in Excel, by comparing the values of GL and YL; $p \geq 0.05$: no statistically significant differences (NS); $p \leq 0.05$: statistically significant difference at 95% confidence level (*); $p \leq 0.01$: statistically significant difference at 99% confidence level (**); $p \leq 0.001$: statistically significant difference at 99.99 % confidence level (***).

In the specialized literature, we have not found comparative studies (still green leaves and yellowed leaves) focused on the behavior of the *P. vera* species during the establishment of leaf senescence. Research has focused on the dynamics of some physiological indicators during the phenological phases (Marino et al., 2018 a), but more specifically the impact of some stress factors on plant morphology and physiology (Mehdi et al., 2011; Esmaeilpour et al., 2016a; Ben Hamed et al., 2023; Ravari et al., 2023). In addition, the possibilities of counteracting the negative effects of the external factors (Marino et al., 2018b; Todros et al., 2021; Marino et al., 2023; Miri et al., 2023), as well as intrinsic ones (e.g., sink-source balance) (Marino et al., 2023) expressed through morphological and physiological changes, have been addressed.

The maximum net photosynthetic capacity (Pnmax), apparent quantum yield (μmol m^{-2} s^{-1}] and, dark respiration (μmol m^{-2} s^{-1}) change drastically between leaf phenological stages (Marino et al., 2018). It turned out to present a similar evolution to that specific to other plant species in general. The Pnmax values were low in young leaves (15.09), while the respiration rate showed higher values (2.86), following that once the maturity phase is reached, the assimilation of carbon registers maximum values (19.39), and the rate of the respiration process decreases (1.45). With the onset of senescence, the efficiency of photosynthesis was markedly reduced (12.78).

Marino et al. (2023) highlights the importance of the source-sink balance, because otherwise when the activity at the sink level is higher, the photosynthesis rate will also be at higher values. However, this can accelerate the leaf senescence, simultaneously with the decrease in the photosynthesis process, which at the end of the vegetation period, for deciduous species, can mean an insufficient accumulation of the reserve compounds, to successfully survive during dormancy and the start of vegetation in the following year.

The comparative studies carried out on *P. vera* and *P. atlantica* exposed to drought and later rehydration highlighted an evident reduction of gas exchange and chlorophyll content in *P. vera*, which indicates a lower degree of its adaptation to drought. At the same time, even if the hydric status after hydration was restored in both species, the physiological indicators such as, net photosynthesis, transpiration rate, and stomatal conductance had lower values than those determined in the well-watered seedlings (Ben Hamed et al., 2023).

To successfully overcome the drought periods, different procedures have been addressed by researchers. Todros et al. (2021) tested different combined water harvesting techniques (mulching × micro-catchment) for *P. vera* in Northern Jordan and found a significant improvement in soil characteristics (e.g., water content) as well as morphological indicators (e.g., plant height, number of leaves on the tree) and respectively, physiological ones.

Furthermore, the behavior of *P. atlantica* and *P. vera* seedlings under conditions of water stress, excess light and temperature proved to be different in terms of relative water content, net assimilation, transpiration, Pn/g_s and Pn/Ci (Ben Hamed et al. 2021). For all these physiological indicators, the values were higher in *P. atlantica*, compared to *P. vera,* and the authors explanation refers to the activation of some internal mechanisms for efficient osmotic adjustment, as well as photoprotection at the level of the photosystem II.

Although pistachio is known to be resistant to soil drought, however *P. vera* cultivars have a different physiological behavior, as highlighted by the studies carried out in the greenhouse by Esmaeilpour et al. (2016 a), by applying two levels of osmotic stress (Ψs=-0.75 and Ψs=-1.5 MPa). In such conditions, the relative water content and the water potential of the leaves decreased significantly compared to the control, while the water use efficiency had higher values, for all the analyzed varieties. No significant differences were recorded regarding the stomata density and their dimensions, even if 'Ohadi' and 'Kaleghochi' had the widest stomata, whereas 'Akbari' had the most stomata number. With good reason, the authors specify the need to carry out some studies in field conditions, to elucidate these aspects.

Also, the studies carried out by Mehdi et al. (2011) in 5-year-old female trees (Mateur variety grafted on *P. vera*) by applying 3 levels of salt stress, highlighted the fact that in the case of the medium salinity level (5 dS/m ECw) the growth in length and number of axillary shoots was stimulated. On the other hand, at the highest used electrical conductivity (EC) value (12 dS/m), the growth indicators decreased significantly, and the content of proline and soluble carbohydrates in the leaves significantly increased. However, no significant differences were recorded relative to the leaf's electrolyte leakage.

The studies carried out by Ravari et al. (2023) on 9 *Pistacia* genotypes under the action of negative temperatures highlighted the variability of the determined indicators (electrolyte leakage percentage, soluble carbohydrates, phenolic compounds) in relation to the species, but what should be highlighted is the fact that *P. vera* var. Sarakhs was on his best behavior.

CONCLUSIONS

The male specimen of *Pistacia vera* L., grown in the Botanical Garden of USAMV Bucharest is a small tree, with simple but also trifoliate leaves.

In the petiole of the leaves and in the epidermises has been observed the unicellular non-glandular hairs.

The vascular bundles in the petiole and median vein are of the collateral type, with secondary structure.

Secretory ducts are present in the phloem.

The leaf is amphystomatic, with stomata of the anomocytic type.

The mesophyll of the leaf is equifacial.

During the autumn senescence, there are major changes in the physiological processes of the leaves, closely interdependent with the growing climatic conditions (characteristic of each year), so that the plants can successfully cross the dormant period and be able to successfully start a new growing season.

Physiological indicators can be useful for the characterization of the pistachio adaptation mechanisms in the resilience integrated framework.

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VALORIZATION OF SWEET POTATO (*IPOMOEA BATATAS* (L.) LAM) AND CUSTOMERS' PERCEPTION ON SOME INNOVATIVE PRODUCTS

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Abstract

Sweet potato (Ipomoea batatas) is a perennial tuber, belongs to the botanical family Convolvulaceae and it is native to Central America. Sweet potatoes are an exceptionally essential crop in several parts of the world, growing well in tropical, subtropical and temperate areas, being produced in more than 100 countries. In different parts of Africa, Asia, and the Pacific. The aim of this study is to present some innovative products with sweet potato and some fruits (jujube, pawpaw, kiwi, apricots, peaches, apple, pears) and customers' perception of this. The sweet potato tubers were provided from SCDCPN Dăbuleni and the other fruits from the experimental field within the Faculty of Horticulture, Bucharest. The products were prepared at the pastry ''Moesis by Angelo", in Tulcea, Romania and in the Integrated Fruit Growing Laboratory, and the tasting was made at the Research Center for Studies of Food Quality and Agricultural Products. Customers' perception consisted on the evaluation of general appearance, color, texture, taste and flavor, noticed with grades from 1 to 7, using a Hedonic scale, and it was made by specialized persons.

***Key words**: cookies, pie, purple sweet potato, Ziziphus jujuba Mill., white sweet potato.*

INTRODUCTION

Ipomoea batatas (L.) Lam, commonly known as sweet potato is a perennial tuber belonging to the family *Convolvulaceae*. (Mohanraj R. and Subha S., 2013; Purseglove, 1972; Woolfe, 1992; Mohammad, 2021).
Flowers can be white or purple, and leaves can be green or purple. Flesh can be white, cream, yellow, orange, or purple (Woolfe, 1992; Bovell-Benjamin, 2007; Burri B. J., 2010). The intensity of the color is attributed to carotenoid content (Ameny et Wilson, 1997; Nungo et al., 2007).
Sweet potatoes grow well in tropical, subtropical, and temperate areas. Originated in the New World, were introduced into Spain, India, and the Philippines by Spanish explorers in the 15th and 16th centuries. Their distribution is now worldwide. (Woolfe, 1992; Bovell-Benjamin, 2007; Burri B. J. 2010).
It is amongst the world's most important, versatile and under-exploited food crops, because it is high yielding and drought tolerant, with wide adaptability to various climates and farming systems with more than 90 million tonnes in annual production, contributed mostly by Asian and African countries, especially China, the leading producer of sweet potatoes at the global level (Diop, 1998; Jiang et al., 2004; FAOSTAT, 2020; Mohammad, 2021).
In **Romania**, the sweet potato is recently cultivated, especially in the South-West region. (Dinu et al., 2021).
Also, sweet potato is a typical food security crop because it can be harvested little by little over several months (Bovell-Benjamin, 2007).
Nutritional composition and health benefits of sweet potato
According to the Food and Drug Administration, a nutrient can be classified as "low source" or "good source" or "rich source" when a food contains 20% of the Daily Value (%DV) of the particular nutrient, respectively (Mohammad, 2021).
The sweet potato has immense potential and has a major role to play in **human nutrition**, food

security, and poverty alleviation in developing countries. (Bovell-Benjamin, 2007).

From a dietary and nutritional perspective, sweet potato (*Ipomoea batatas* L. Lam) is a good source of the basic nutrients and different vitamins, minerals, antioxidants and bioactive compounds or polyphenols (present in Table 1) (Burri, 2011; Satheesh and Solomon, 2019; Alam et al., 2016, 2020; Islam, 2006, 2014; Sun et al., 2019). β-Carotenes are important pigments in sweet potato roots as provitamin A precursor, which is essential for human health (Low et al., 2017; Mayne, 1996; Teow et al., 2007; Huang et al., 2007; Mark et al., 2009; Rosero et al., 2022.

Sweet potatoes contain oxalic acid, a naturally-occurring substance found in some vegetables which may crystallize as oxalate stones in the urinary tract in some people (Faboya et al., 1983; Mohanraj et al., 2013). Because of its proven anti-ulcerative activity, it could be considered when treating gastric ulcers (Rengarajan et al., 2012; Mohanraj et al., 2013). Compared to major commercial vegetables such as spinach, broccoli, cabbage, lettuce, etc., sweet potato contains high concentrations of fiber, minerals, polyphenolics, anthocyanins. These are claimed to have antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, cardioprotective, antimicrobial, immune system enhancing, cardiovascular effects, and hepatoprotective properties (Mohammad, 2021). Nutritional composition and properties are presented in Table 1 and also in Figure 1.

Table 1. Nutritional value of sweet potato, cooked, baked in skin, flesh, without salt

Nutritional value per 100 g		Vitamins (per 100 g)		Minerals (per 100 g)	
Energy	90 kcal	**Vitamin A**	961 µg	**Calcium (Ca)**	38 mg
Carbohydrates, by difference	20.7 g	**Thiamine (B1)**	0.107 mg	**Iron (Fe)**	0.69 mg
Fat	0.15 g	**Riboflavin (B2)**	0.106 mg	**Magnesium (Mg)**	27 mg
Protein	2.01 g	**Niacin (B3)**	1.49 mg	**Manganese (Mn)**	0.497 mg
Ash	1.35 g	**Vitamin B6**	0.286 mg	**Phosphorus (P)**	54 mg
Water	75.8 g	**Vitamin C, total ascorbic acid**	19.6 mg	**Potassium (K)**	475 mg
Fiber, total dietary	3.3 g	**Vitamin K**	2.3 µg	**Sodium (Na)**	36 mg
Sugars, total including NLEA	6.48 g	**Betaine**	34.6 mg	**Zinc (Zn)**	0.32 mg
Starch	7.05 g	**Carotene, beta**	11500 µg	**Copper (Cu)**	0.161 mg
Fructose	0.5 g	**Carotene, alpha**	43 µg	**Selenium (Se)**	0.2 µg
Sucrose	2.28 g				
Maltose	3.12 g				
Glucose	0.57 g				

Source: USDA, 2019

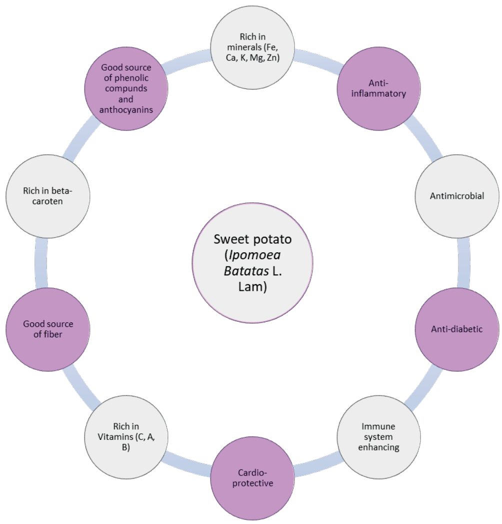

Figure 1. Nutritional composition and health benefits of sweet potato (*Ipomoea batatas* (L.) Lam)

MATERIALS AND METHODS

The aim of this study is to present some innovative products with sweet potato and some fruits and customers' perception of this. The sweet potato (*Ipomoea batatas* (L.) Lam) tubers were provided from SCDCPN Dăbuleni and the other fruits from the experimental field from the experimental field within the Faculty of Horticulture, Bucharest.

The products were prepared at the pastry ''Moesis by Angelo", in Tulcea, Romania and in the Integrated Fruit Growing Laboratory.

The raw material used for the products was: boiled and grated **white and purple sweet potato**; fruits: jujube (*Ziziphus jujuba* Mill.) (dehydrated diced and powder), pawpaw (*Asimina triloba* Dunal), kiwi (*Actinidia deliciosa*), banana, apricots (*Prunus armeniaca*), peaches (*Prunus persica*), apple (*Malus domestica*), pears (Pyrus); cocoa; honey; sugar; white and black chocolate; cheese (Figures 2-5).

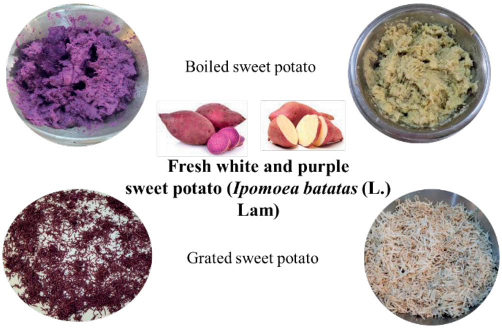

Figure 2. Sweet potato (*Ipomoea batatas* (L.) Lam) white and purple

The products that were made:

PIE with:

- grated white sweet potato
- grated purple sweet potato
- boiled white sweet potato and cheese
- boiled purple sweet potato and cheese
- boiled white sweet potato and jujube
- grated white sweet potato and powder of jujube
- grated purple sweet potato and powder of jujube
- boiled white and purple sweet potato and sugar
- boiled white sweet potato and ground walnut

Figure 3. Pie with white and purple sweet potato

Cream with:

- white chocolate and boiled **white sweet potato**
- white chocolate, **white sweet potato** and **pawpaw**
- white chocolate and boiled **purple sweet potato**
- black chocolate with milk and boiled **white sweet potato**
- black chocolate and boiled **white sweet potato**
- white chocolate, boiled **purple sweet potato** and **pawpaw**

Figure 4. Cream with white and purple sweet potato

Boiled purple sweet potato with:

- kiwi
- apple
- pears
- banana
- cocoa and honey
- apricots
- peaches
- pawpaw

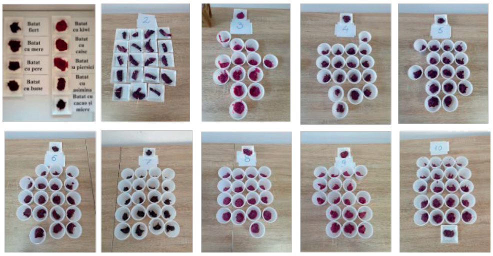

Figure 5. Boiled purple potato with fruits

The combinations of sweet potato and other fruits was to see how they look together and that is the most appreciated variant.

RESULTS AND DISCUSSIONS

The tasting was made at the Research Center for Studies of Food Quality and Agricultural Products, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Figure 6).

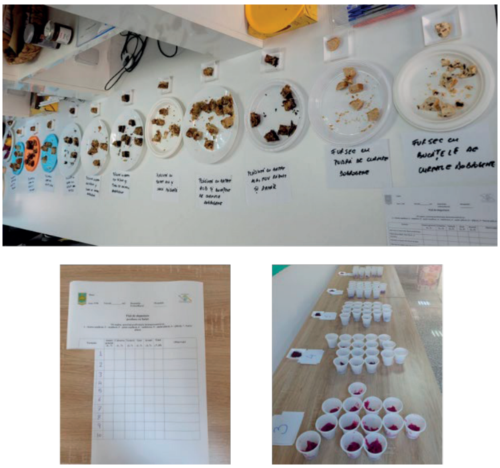

Figure 6. The products testing

Customers' perception consisted on the evaluation of general appearance, color, texture, taste and flavor, noticed with grades from 1 to 7, using a Hedonic scale, and it was made by specialized persons.

The results show us that:
The difference between **grated and boiled sweet potato** is that grated potatoes oxidize much faster.

The pie with white sweet potato and diced **jujube** was the most appreciated among the 9 recipes maybe also for the aroma given by the white potato in combination with the natural taste of sugar from jujube (Figure 7).

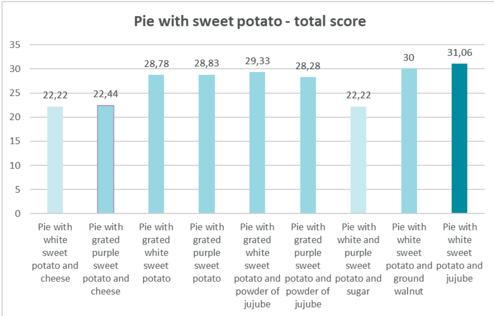

Figure 7. Pie with sweet potato – total score

The combination of **purple sweet potato**, cocoa and honey was also very appreciated and in the case of combination with **kiwi fruits**, the color was very interesting because kiwi has a high vitamin C content and the resulting color was pink (Figure 8).

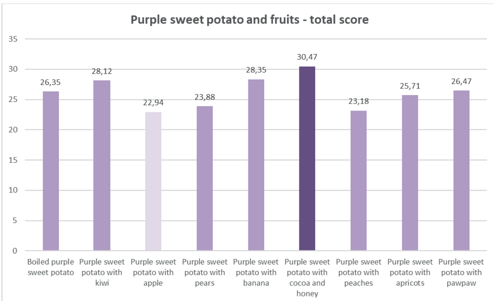

Figure 8. Purple sweet potato and fruits – total score

CONCLUSIONS

We **concluded** that: **sweet potato** (white or purple) is an important base in the preparation of desserts, due to the **color** it gives to the product and its nutritional properties but and jujube being a perfect substitute for the sugar, because it has a very **high nutraceutical value**, beside the sweetening strength.

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WATER IN BIOLOGICAL SYSTEMS - A CHALLENGE FOR PLANT PHYSIOLOGY AND BIOPHYSICS WITHIN A CLIMATE CHANGE PERSPECTIVE

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Abstract

As the world handles with the multifaceted challenges of climate change, understanding the profound implications of water in plant system is of high interest. The aim of this study was to explore the challenges posed by water in plant physiology under climate change context. Three searches were performed in the Web of Science database using three combination of key words "water and plant physiology", "water and biophysics" and "water and climate change". Full records were assessed following author keywords and keywords plus. The database was refined by quick filters "review articles" from the last 5 years. The results from the first search highlight drought, in a share of 16%, as the most studied abiotic stress on plants (32%) and overall stress (30%), root zone (13%) and salinity effects within 13%. The second assessment brings an increased interest upon mathematical modelling (26%) and different elements dynamics (17%). The third specific search points out the present interest in genetic analysis (18%) for assessing stress tolerance (12%) that impact plant physiology (6%), stomata (6%) and chlorophyll content (3%). The perspectives for future research must be concentrated to increase especially the fields with low percentages.

***Key words**: abiotic stress, crop growth, dynamics, seed, stress response.*

INTRODUCTION

Water, so called "life matrix", is the most encountered substance on our planet, and represents an essential element that sustains all living organisms (Ball, 2008). Its significance cannot be overstated, as it serves as the foundation upon which life as we know it is built (Rathi et al., 2021). As our world encounters more and more often disruptive forces of climate change, the intricate interplay between water and biological systems has become increasingly critical to our understanding of the natural cycles. Water key parameters and relations in plants and soil water availability are close connected with global climate change (Grossman, 2023). The influence upon these comes first of all from the regional patterns regarding precipitation (Feller et al., 2017).

The effects of climate change are clear in various forms, from rising temperatures and shifting weather patterns to more extreme and unpredictable events such as droughts, floods and false springs (Bartok et al., 2021; Bagheri et al., 2023; Grossman, 2023). These changes have a profound impact on ecosystems, particularly in the context of plant physiology and biophysics. Plants, which play a pivotal role in the Earth's ecosystems, are linked to water availability (Ball, 2008; Lambers el al., 2019). They serve as the primary converters of sunlight and carbon dioxide into the energy that fuels life, a process that relies heavily on water as a crucial ingredient (Aresta and Dibenedetto, 2021). The assumption that life could not exist without water is empirical because the only general prove is that an environment could not sustain live without liquid water (Ball, 2008). Within the broader spectrum of climate change, understanding the role of water in plant physiology and biophysics is of high importance. Water regulates the opening and closing of stomata (Fanourakis et al., 2020), the small pores on plant leaves that enable gas exchange, including the crucial intake of carbon dioxide for photosynthesis (Leakey et al., 2019). It also acts as a transport medium for

nutrients, minerals, and other essential compounds within the plant (Yadav et al., 2021).
The relationship between plants and water is not one-sided. It is deeply linked and intersected with the broader environment. The availability of water resources directly affects plant distribution, growth, and survival, and it has waves effects on the entire ecosystems (Rossati, 2017). Climate change introduces a new layer of complexity, as shifting precipitation patterns, prolonged droughts, and increased evaporation rates it poses different threats and challenges to the delicate equilibrium that has evolved over time (Ostmeyer et al., 2020).
In this context, the interconnected effects between water in the biological systems especially on plant functioning and physiology, holds great significance. This complex relationship between water, the environment, and plant science was taken into different studies (Lambers el al., 2019; Snyder et al., 2022; Dickman et al., 2023). The exploration of all representative studies could offer the opportunity to unlock the interest of scientists toward water role in shaping life as a unique perspective between biology and climate change.
This review aim was set to explore the challenges posed by climate change particularly in the context of plant physiology but also for a better understanding of potential solutions for mitigating its impact on biological systems. By exploring the multifaceted implications of water in the current context of a changing climate, we can highlight knowledge and insights that are crucial for the preservation of several ecosystems and the well-being of all living organisms.

METHODOLOGY

The Web of Science Core Collection–WOS database was interrogated and accessed on 25 October 2023 using three keywords in a two by two combination (Figure 1).
First search was performed with keywords combination „water and plant physiology" and resulted in 6626 articles. Then, they were first refined with quick filters by review articles in a number of 721 and the last filter applied was publication year, only the articles from the last 5 years (2019-2023) were selected and resulted in a final number of 298 reviews.
The second search was done with the keywords combination "water and biophysics" and resulted in 898 articles. All were first refined by quick filters only into reviews and there were 110 in number. The second refining following the last 5 years of publication was applied and it was obtained only 23 review on this topic combination.
The third search was made according keywords combination "water and climate change". A total of 143562 results were found, from which 9442 were review articles of which only 4168 were published in the last 5 years. The results were then refined following Web of Science Categories into Plant Sciences and it were extracted 378 reviews.
After applying all the filters and the number of reviews were refined, three excel files were downloaded with full records on all final selected documents. All data bases were further analysed based on the author keywords and keywords plus. Essential information was summarized and highlighted from all the records consulted. A *prisma diagram* was compiled to highlight the methodology used for refining the information consulted (Figure 1).

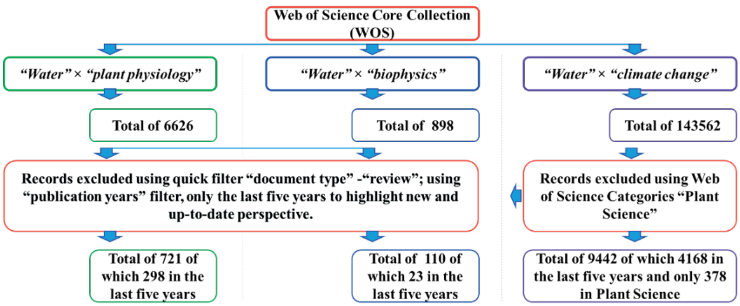

Figure 1. Prisma diagram representative for the methodology of consulted data base

RESULTS AND DISCUSSIONS

Overview on "water and plant physiology"
Regarding the results obtained from the first search in the data base, the highest percentage within author key words from this combined fields is represented by plants (32%) (Figure 2). The following area of interest was drought with 16%. This particular abiotic stress is produced in the present mainly because of extreme heat events and climate change (10%) (Da Costa et al., 2021; Abdelhakim et al., 2022; Grossman, 2023). Other type of abiotic stresses and pollution with heavy metals represents <10% of the studies regarding author key words. Bioremediation of heavy metals is essential for human wellbeing and plant physiology. Some plant species were included in the category of heavy metal absorbers for example, *Helianthus*, *Hibiscus*, *Allium* and *Eucalyptus* (Vaid et al., 2022). Phenotyping, phenology (York, 2019) and stomata (Misra et al., 2019) altogether within plant physiology domain represent a slightly low share of 6% (Chen et al., 2022). Nano-science and carbon dynamics keywords were used in less than 5% from the total of the most ten used **author key words** from the selected data base (Maswada et al., 2020; Irshad et al., 2021; Venzhik and Deryabin, 2023).

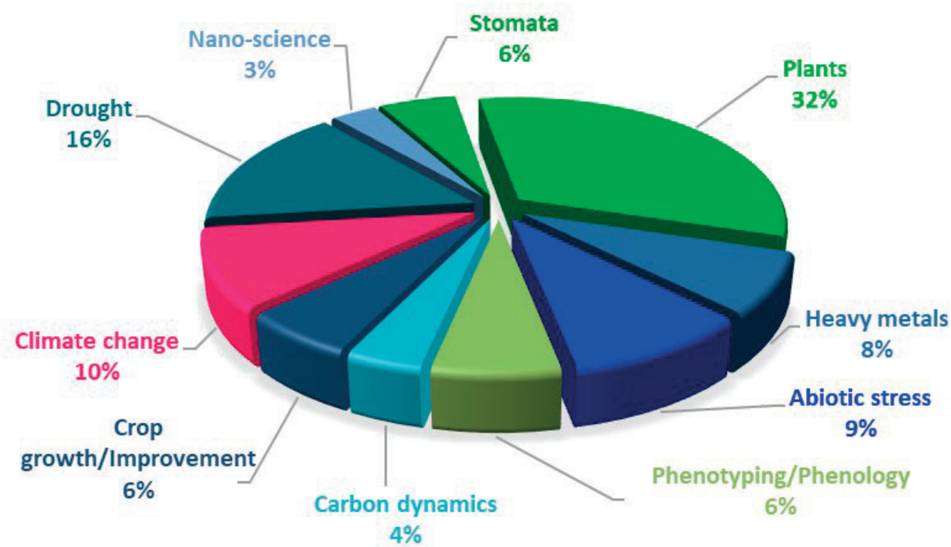

Figure 2. Principal selected author key words in percetages to highlight the importance of key words combination "water and plant physiology"

All physiological activities of the plants depend on water. Therefore, water is essential for all living organisms because is the main component of the cells (Rathi et al., 2021). The water properties are responsible for many plant functions - uptake, transport and transpiration (Lambers et al., 2019; Scharwies and Dinneny, 2019) (Figure 3).
Its distribution varies within a plant (Lambers et al., 2019). Woody plants contain low water content and other parts contain higher water content. Water represents a medium in which solutes diffusion happens through plant cells (Bhatla et al., 2018). Acts as a solvent for plant nutrition. Soil mineral nutrients from the soil solution are absorbed into the plant and transported to each cells assuring turgidity (Jones, 2012). Water sustains many biochemical reactions. An optimum water supply in plants assures and regulates the mechanical support and the rigidity to the plants. Water content is influencing plant movements. Plants adapted and developed a waxy cuticle to control and limit the water loss (Scharwies and Dinneny, 2019).

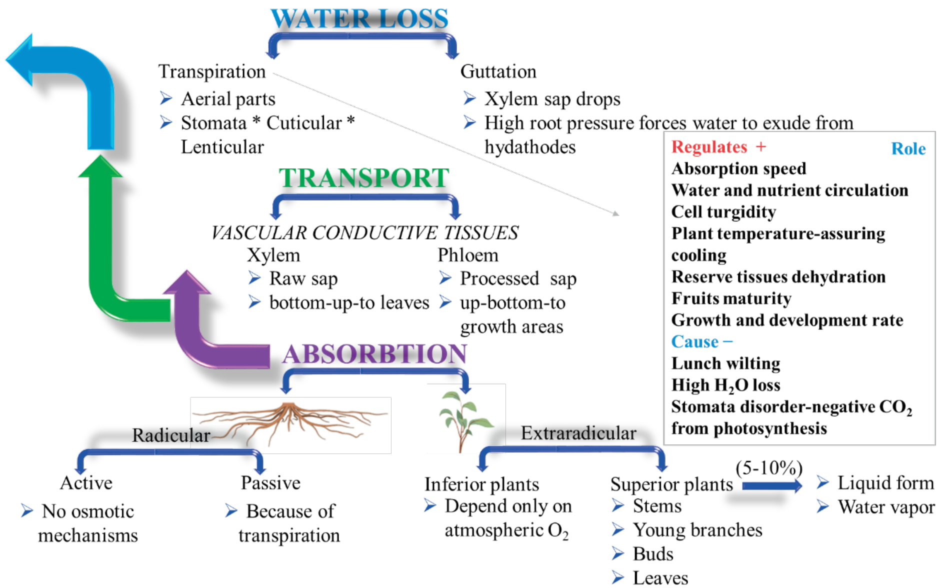

Figure 3. Plant water regime respectively uptake throughout absorption, nutrients transport and water loss ways

Low water content produce leaf folding of sensitive plants, reduce opening or closing of the flowers and of leaves stomata with direct role in gas exchange. Plant growth elongation depends on water availability. Water in photosynthesis represents the principal source of O_2. Plant heat is also regulated by water content. Water represent the medium for dissolving the fertilizers, mandatory for the foliar types. Some spores, fruits and seeds depend on water to spread and multiply. A seed appears dry, however to be viable it still must contain water. All terrestrial plants consume high water amount each day, increasing their transpiration rates throughout evaporation from the stomata leaves. Altogether certify the decisive role of water that can limit plant growth, development and productivity for all environments.

The key words plus emphasize several stresses (Vincent et al., 2020; Dalal, 2021; Venzhik et al., 2021) as being with high interest in the last five years' research field with 30% from the total ten selected keywords (Figure 4). A very specific type of stress, salinity (Javed et al., 2019), was very intensively studied with 13% share together with plant roots morphology (Arif et al., 2020). Other plant physiological parameters were mention in the scientific papers like photosynthesis (12%), stomata (8%), oxidative stress (8%) and osmotic adjustment (4%). Several essential physiological processes like photosynthesis and respiration are influenced by the electron transfer between proteins and different other biomolecules (Ball, 2008). A very low share only of 4% was formed by the keywords plus growth promoting rhizobacteria, plasma membrane and a plant test *Oryza sativa* L.

Osmotic stress tolerance can be faced by inducing a particular physiologic state starting with seed treatments previous germination. Seed priming represent a traditional physiologic method with many proved advantages (Paul et al., 2022). Seed priming emerged for obtaining equal seedlings, controlled growth and development. The seedlings obtained by primed seeds were resistant to abiotic and biotic stress.

To comply with the new agenda of SDGs (Sustainable Developmental Goals), SDG 2, SDG 12 and SDG 15, agriculture must be resilient and sustainable (Stoian et al., 2018; Pop-Moldovan et al., 2022) and within this purpose seed priming could assure a useful crop starter tool. In the context of plant stress physiology, optimizing plant physiology function by means such as seed priming is indeed a powerful, cost-effective and efficient tool (Vincent et al., 2020).

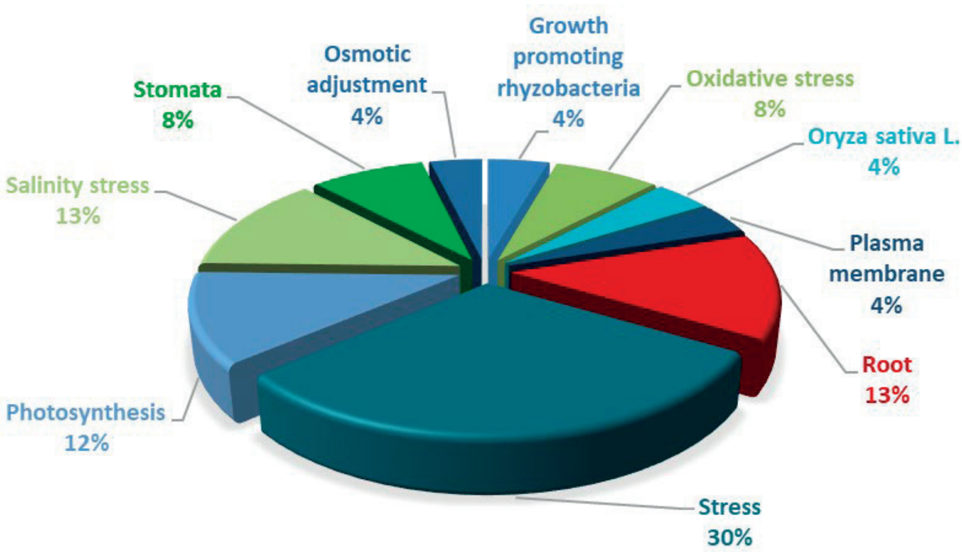

Figure 4. Principal selected key words plus in percetages to highlight the importance of key words combination "water and plant physiology"

Hydropriming pre-sowing technique, implies seed imbibition enough for enzymes activation and necessary for germination however insufficient and without germination to take place (Paparella et al., 2015). The imbibition is followed by forced dehydration suspending the seeds. Altogether induce an optimization of seeds imbibition in the field condition, uniformity at emergence, and strong vigorous seedlings and with high degree of resistance to pest and diseases (Jisha and Puthur, 2018).

Interest regarding "water and biophysics"

Author key words and key words plus are very important and offer an overview of different research fields connected to biophysics and water. Usually, independent of the obtained results, setting up models and mathematical (Wade, 2020) aspect in the most frequent accessed domain (26%) toward explaining different patterns (Figure 5). The use of biophysics methods and technologies often determines some cell wall parameters (Yoo et al., 2019), hereby cell biology (Vlasov et al., 2020; Theillet and Luchinat, 2022) stand together with the same percentages of 15%. With a medium percentage of 10% representing one of the most important trends in the present it could be found different molecular simulation (Cerutti and Case, 2019; Filipe et al., 2020) and the use of laser for different purposes (Satta et al., 2022).

In lower range percentage between 3-8, different aspects were assessed like seed aging (Nadarajan et al., 2023), membrane (8%) (Sanders, 2019; Corin and Bowie, 2022), fluorescent membrane (Filipe et al., 2020), molecular dynamics by X-ray diffraction (Cerutti and Case, 2019) and fluorescent RNA aptamer together with assessing solvation dynamics 5% (Cao et al., 2021).

Plant genetic resources conservation is necessary to avoid losing important species with different site specific adaptation qualities (Nadarajan et al., 2023). The studies concluded that seed longevity it could be influenced by many factors grouped by two categories, internal features and external environment. The internal features refer to chemical composition and structure and the external storage conditions are related with temperature, humidity and oxygen. Seeds aging can be assessed throughout different analysis such as physiological, cytological, physico-chemical, molecular and even genetic (Nadarajan et al., 2023).

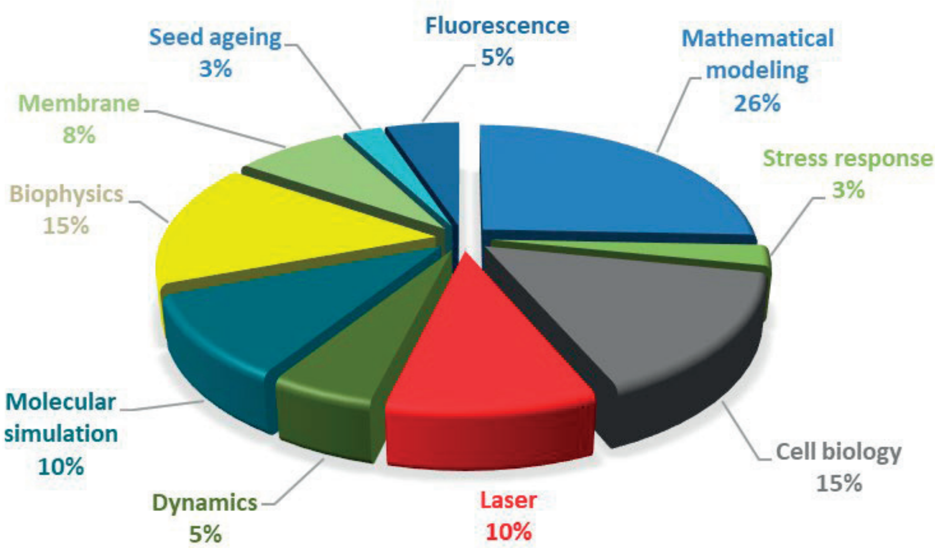

Figure 5. Principal selected author key words in percetages to highlight the importance of key words combination "water and biophysics"

The Nuclear magnetic resonance (NMR) (Figure 6) spectroscopy represents a powerful technique for assessing mobility, structure and water molecules movement in various biological systems (Kumar et al., 2022). The NMR represents the most extensively used method of assessing the water properties in biological systems (Cooke and Kuntz, 1974).
Dynamics and protein (17%), assessments were made in the last 5 years especially on the cell level. Continuous degradation of proteins, lipids and nucleic acids, are damaging the cell constituents (Nadarajan et al., 2023). The disordered proteins were used to protect the cellular environment, after exhibiting some dynamical and structural reordering (Romero-Perez et al., 2023). Therefore, protein folding is a fundamental process of life with important implications throughout biology (Corin and Bowie, 2022). Between 2011-2020, molecular dynamics (MD) simulations were studied for all major classes of fluorescent membrane probes. The cell was assessed in a share of 11% using RNA (Cao et al., 2021), DNA, Raman spectroscopy- RS (Vlasov et al., 2020) and NMR, analysis (Theillet and Luchinat, 2022). Also in the study context of bilayer-inserted fluorescence probe behavior (Filipe et al., 2020), the lipid membranes have shown 10% interest. Interest regarding keywords plus keywords NMR and X-Ray analysis was 15% respectively 10%. Since its beginnings (1950) NMR spectroscopy has been applied to cells and tissues analysis and generated valuable knowledge about this subjects (Theillet and Luchinat, 2022). X-ray crystallography has made possible the current knowledge of the structures of biological macromolecules (Cerutti and Case, 2019). Interest for Raman spectroscopy was 6%, this analysis makes important contributions to the study of structural biology, biophysics, cells, and tissues imaging towards development of various medical diagnostic tools (Vlasov et al., 2020). Low percentages interest regarding keywords plus data were seed and RNA of 4%. The emerging development of fluorescent RNA aptamers and their potential application in live cell imaging was followed (Cao et al., 2021). The evolution of knowledge on seed longevity over the last five decades in terms of seed aging mechanisms were also assessed. Seed longevity represents a complex trait and varies greatly between species and even seed lots of the same species (Nadarajan et al., 2023). Also three type of stresses were assessed respectively osmotic (Romero-Perez et al., 2023), oxidative (Yoo et al., 2019) and drought stress (Boursiac et al., 2022).

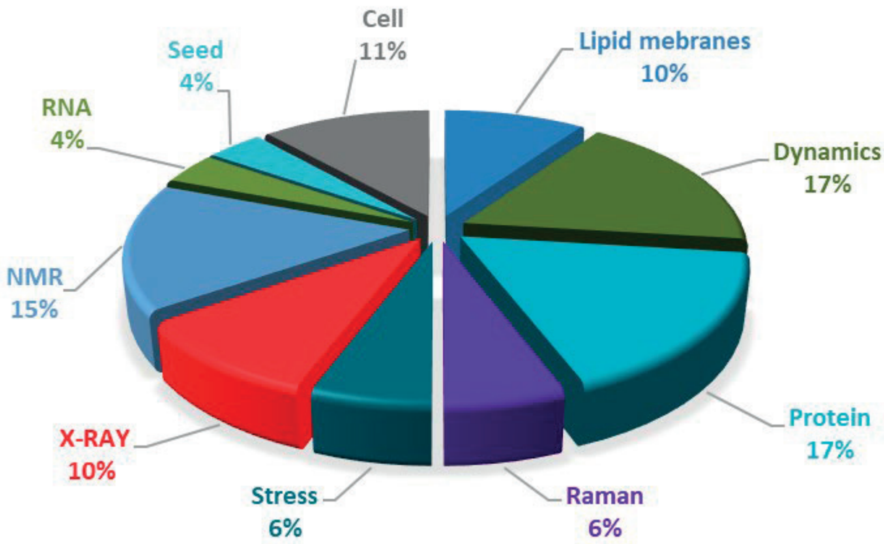

Figure 6. Principal selected key words plus in percetages to highlight the importance of key words combination "water and biophysics"

The dipole-dipole interaction in a water molecule between two protons is a cause of the Brownian rotations. Therefore, the fluctuations produce the most important mechanism of proton relaxation in liquid phase of water (Cooke and Kuntz, 1974). The water interactions strength is in the following order water and anions, water and cations and water and dipole (Cooke and Kuntz, 1974).

At a larger scale, several emerging technologies could be used to fill gaps between quantitative, morphologic and functional measurements to highlight different plant stresses. These emerging technologies could refer to nuclear, medical physics like computer tomography, positron emission tomography or magnetic resonance imaging (Galieni et al., 2021).

Studied trends regarding "water and climate change"

Omics approaches (Da Costa et al., 2021) represent quantitative methods such as metabolomics, genomics (Matallana-Ramirez et al., 2021; Singh et al., 2022), phenomics (Roitsch et al., 2022), nutrigenomics (Fischer and Efferth, 2021), proteomics (Singh et al., 2022) and offer very sensitive parameters stress dependent, representing a dynamic disruptive approach (Galieni et al., 2021).

The cell walls structure could present some microbarriers that influence the water diffusion (Figure 7). These microbarriers from the cell membrane has a length between 1-10 µm, these could be measured by pulsed NMR. Because of the role of these barriers decrease the intracellular water coefficient of diffusion, and the measurement could be done by a magnetic field gradient over a time range (Cooke and Kuntz, 1974). Further, analyzing **author keywords** from the third search (Figure 7), it can be synthetized that the climate changes have the highest share of 34%, and plant responses to abiotic stresses (16%) such as drought, salinity as well as extreme temperatures are of high interest (Ma et al., 2022; Shelake et al., 2022). These factors cause plants a certain degree of stress tolerance (12%) quite significant (Khadka et al., 2020; Rosero et al., 2020).

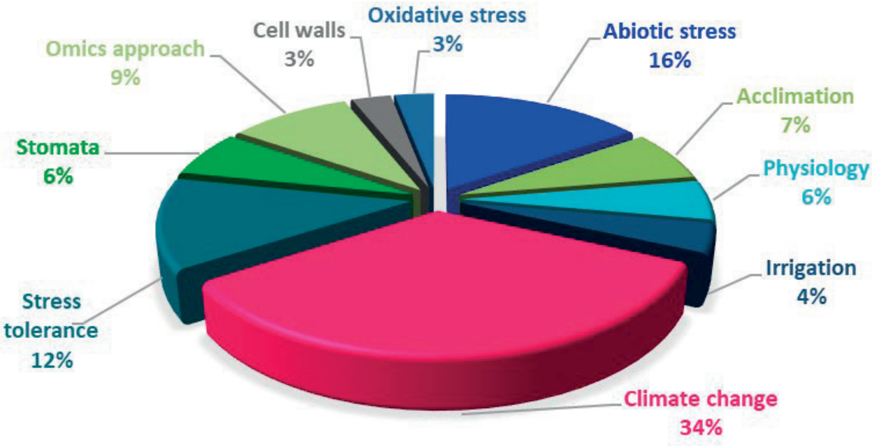

Figure 7. Principal selected author key words in percetages to highlight the importance of key words combination "water and climate change"

Newest initiatives of assessing stress tolerance are represented by Omics with 9% (Baslam et al., 2020; Kapazouglou et al., 2023). Here comes acclimatization with a percentage of 7% which presents its advantages and disadvantages, observed primarily in the physiological properties of plants (Grossman, 2023). A parameter of interest evaluated in plant physiology (6%) would be the stomata which has a percentage of 6%, similar to the percentage in Figure 2 (Driesen et al., 2020; Buckley et al., 2020; Han et al., 2021). Moreover, irrigation (4%) can have an important impact on the structure of cell walls (3%) and adaptation through mechanisms resistant to oxidative stress (3%) (Marc et al., 2022; Atta et al., 2023; Pais et al., 2023).

The highest share obtained after the third search "water and climate change" among the keywords plus (Figure 8) was climate change with 22%.

Climate change is a challenging issue for habitats (Noun et al., 2022; Soares et al., 2022; Oneț et al., 2024) and has a major impact on water availability (Hajek and Knapp, 2022; Lin et al., 2023). This has direct implications for the development of genetic analyses (18%) (Hyun, 2020; Trono and Pecchioni, 2022) and for determining the effects of drought stress (17%) (Serna, 2022; Yang and Qin, 2023) and salinity (15%) (Ahmad et al., 2022; Kumari et al., 2022; Khalid et al., 2023) on plants. The assessment of root development (7%) (Calleja-Cabrera et al., 2020; Hill et al., 2021; Heredia et al., 2022) for several plants, and the resistance of plants to various stressors signalled via abscisic acid (7%) (Habibi et al., 2023) are the focus of recent relevant research in the field. Abscisic acid, a hormone synthesized by plants in the presence of abiotic stress (5%) (Kaya et al., 2009), can counteract the negative effects on the vegetal organism (Li et al., 2020). A low share of 3% is representative of different analyzed parameters such as chlorophyll content and greenhouse gases (GHG). Climate change has occurred as a consequence of the global atmosphere deterioration caused by the elevated levels of GHG emissions (Engonopoulos et al., 2021). Chlorophyll content represents an indirect parameter of plant physiological processes function (Bhardwaj et al., 2021; Ahmad et al., 2022). Arbuscular mycorrhizal fungi (3%) can also decrease the harmful effects of some abiotic stresses through their nutrient supply (Zou et al., 2021; Corcoz et al., 2022). Moreover, these symbiotic interactions imply sustainable and eco-friendly alternatives in agricultural strategies supporting food security (Vega et al., 2021; Stoian et al., 2022).

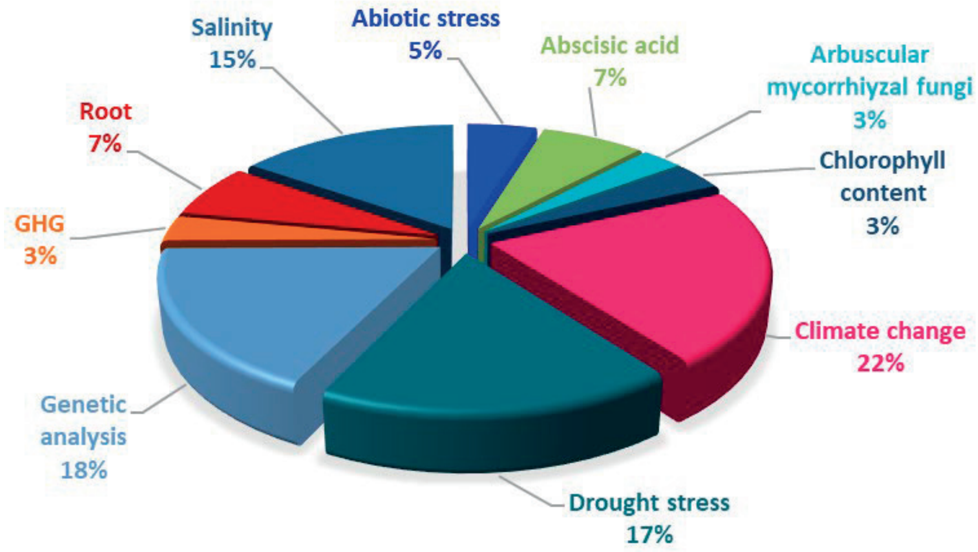

Figure 8. Principal selected key words plus in percetages to highlight the importance of key words combination "water and climate change"

CONCLUSIONS

This study provides scientometric analysis insights within the water essential role in plant physiology connected with biophysics from the Web of Science database.

The last five years' studies were concentrated mainly to assess different stress (osmotic, oxidative and drought) response mechanisms in plants with help of genetics and chemistry analysis.

The plant physiology field needs to be intensively studied to increase the studies range of 3-6% with future studies on chlorophyll content change with climate change and different applied stresses together with detailed stomata assessments.

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NETTLE PLANTS FINGERPRINT BASED ON XRF ANALYSIS

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Abstract

Stinging nettle (Urtica dioica L.) is a perennial crop well adapted to a varied range of environmental conditions. The cultivation of nettle could help meet the high demand of nutritious plants, for food, nutraceuticals and as a substitute for artificial fibers for different industries. Nettles are well known as hyperaccumulators of heavy metals, growing as weed, worldwide, which makes them suitable for the present study. The aim of this study is to present the possibility of using X-ray fluorescence (XRF) spectrometry as a valuable tool to create fingerprints to evaluate the environmental pollution of a specific area using soil and nettle plants samples, as well as verifying the quality of plants utilized as potential food sources. Nettle plants and soil samples were randomly collected from different areas of Romania, dried under controlled environment, then prepared for XRF Analysis. All samples were analyzed in triplicate using Hitachi X-MET8000 XRF analyzer and the results were statistically evaluated using statistical programs. The results show the possibility to obtain valuable fingerprints based on the elemental composition correlated to the geographical origins of plants.

Key words: *Urtica dioica, heavy metals, food safety, mathematical models.*

INTRODUCTION

The distribution of mineral elements in the ecological environment is irregular, having major changes in the structure and content of mineral elements in water, soil and air (Franke et al., 2005).
The X-ray Fluorescence (XRF) technique presents a strong reliability in the identification of chemical elements existing in soil and plant samples, regardless of the instrumental conditions and the type of analyzed plant sample (roots, stems or leaves) (Panebianco et al., 2023).
The characterization of trace elements in natural and environmental samples are frequently made by using conventional methods, i.e., atomic spectroscopic techniques, including ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy), ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) (Panebianco et al., 2023).
The term analytical fingerprint refers to scientific techniques that are used as a traceability tool to identify the source or origin of the products of interest (Watzel, 2023).
The stinging nettle (*Urtica dioica* L., *Urticaceae*), a widespread weed plant - prominent in desert areas, with unpleasant hairs on stems and leaves, is defined by significant economic potentials, being used in human nutrition and medicine since Antiquity. (Rafajlovska et al., 2013, Kavalali, 2003). Due to the existence of some precious compounds, such as proteins, macro and micro elements, tannins, flavonoids and fatty acids, phenolic components, chlorophylls the nettle plants and /or parts are used in several ways (Rafajlovska et al., 2013). Nettle leaves show high levels of vitamins (A and C) and minerals (iron, calcium, manganese and potassium) (Bordean, 2012).
The "fingerprint" of micro-chemical differences suggests a reference for the traceability of the origin of products/plants due to the elemental structure which varies between nettles originated from different places. The detailed characterization of the multi-mineral elements of the plant materials suggests a possible link for the research of the traceability of other valuable products of geographical indication (Xue et al., 2022).

Chemical fingerprints of plants, measured in general concentrations, contribute to research in a number of environmental, biomonitoring and biological analysis sectors (Djingova et al., 2004). Founded in 1990 by O'Byres and Wackernagel, the ecological footprint has the special meaning of combining data from several sectors, expressing them in the form of an equation with four variables (Odagiu et al., 2012).

Heavy metals represent a massive category of genotoxic environmental contaminants that present a serious danger to the environment and human health.

The mineral nutrient content in plant foods is influenced by the plants' capacity to absorb and integrate specific minerals from their growing environment. The term "concrete mineral nutrients" implies particular and well-defined minerals that the plants assimilate based on the conditions of the growing climate.

The essential source of mineral nutrients for plants is the soil, which contains important nutrients for plant growth, such as nitrogen, potassium, phosphorus, magnesium, calcium, sulfur, iron, zinc, copper, manganese, boron, molybdenum, chlorine, nickel (Kirkby, 2013).

The aim of this study is to present the possibility of using X-ray fluorescence (XRF) spectrometry as a valuable tool to create fingerprints to evaluate the environmental pollution of a specific area using soil and nettle plants samples, as well as verifying the quality of plants utilized as potential food sources.

MATERIALS AND METHODS

Collection and preparation of samples

The nettle plant and soil samples were collected during autumn 2003, from Romania: Turcinesti - Gorj county, Vadu-Dobrii - Hunedoara, Bazos, Recas - and Timisoara, Timis county, as presented in Figure 1.

The location L3 is close to the Dendrological Park, located in a grand secular oak forest, which received the status of a protected area for the protection of the biodiversity of the Geno fond and Eco fund since 1994 (Wasicsek A., 2020).

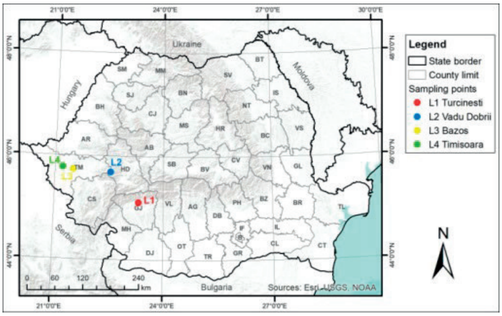

Figure 1. Collection areas of soil and nettle sample

The 5 random selected nettle plants together with the soil samples were collected from each mentioned location (L1-L4). All sub-samples were mixed to create the laboratory soil samples and laboratory nettle samples. The soil samples were sieved through a 2 mm sieve to remove non-soil material, including plant fragments, then homogenized using a grinder, dried under controlled environment and stored in tight sealed containers.

The plant samples were carefully washed with tap water to remove dust and soil particles, followed by rinsing with distilled water to ensure that any residual minerals from tap water were eliminated. This was done to reduce the risk of contamination and maintain the integrity of the samples. The soil was carefully shaken from the roots. Roots were separated from plant stems and leaves. All plant parts were air-dried and homogenized by grinding in a laboratory mill designed for processing food samples.

Samples analysis

The mineral content of soil and plant samples was analyzed using XRF Hitachi XMET8000 portable spectrometer, which can be used to measure a wide range of atomic elements, from sodium (Z11) to uranium (Z92), while providing elementary detection limits from small parts per million (ppm) by weight high percentage (% weight).The XRF method was selected because XRF (X-ray fluorescence spectrometry) is an essential evaluation method of soil and plant samples at medium air pressure, frequently used for qualitative and quantitative analyses (Yonehara et al., 2010). XRF spectrometry is beneficial because it provides the concentrations and presence of chemical elements quickly, with high accuracy (Panebianco et al., 2023).

Statistical analysis

PAST and MVSP programs were used for statistical analysis and mathematical modeling.

Cluster analysis is a method that has the role to identify groups (clusters) or patterns in a set of data on the basis of their similarities or differences. Correlation is a statistical tool used to assess the degree of association of two quantitative variables measured in each member of a group (Aggarwal & Ranganathan, 2016).

Generalized linear models (GLM) is a more flexible form of linear regressions (Guo, 2022), providing a common approach to a broad range of response modelling problems (Faraway, 2010).

RESULTS AND DISCUSSIONS

XRF technique is of great help to obtain fast and reliable informations regarding the elemental composition of the investigated samples. The results are presented in Table 1.

Table 1. Mineral composition of nettle plants and soil samples

Element/S	L1S	L1P	L2S	L2P	L3S	L3P	L4S	L4P
K	18067	67624.33	15521	53359.33	14469	66529.33	16448	45271.67
Ca	7642	54660.67	6923	36390.67	5725	55785.67	47340	46191.67
Fe	27183	1437.67	74907	1361.67	39425	2282	38983	715
Mn	900	206.5	2609	335	491	198.67	973	130
Ti	4225	234.5	8360	133.67	5084	230.67	4699	63
Cu	17	29	56	39	24	20.67	51	1
Mo	0	8	5	6.5	0	7	3	6.33
Zn	72	65	247	74.67	105	61.33	207	23.67
Ni	26	23	94	19	52	19	61	2
Co	43	0	0	0	0	0	40	0
Ba	556	162	657	128	344	122	621	0
Zr	295	10.5	335	6	251	7	335	0
Sr	151	161.33	100	39	153	128.33	192	57.5
Rb	119	9.33	197	91.33	126	6.33	123	3.5
Cr	47	15	141	18	78	12	87	0
Sn	45	42	48	0	39	35	40	0
Ta	32	19	47	20.67	31	18	28	17.5
Th	13	0	16	5	14	0	15	0
Sb	0	19	0	12	0	19.5	0	18.5
Hg	8	5	9	5.5	6	6	11	7
Sc	0	315.5	0	259	0	259.67	161	237.33
Pb	32	0	57	4	31	3	53	0
Cd	0	17	0	0	8	0	0	0

Legend: L1P- location 1 plant, L2P-location 2 plant, L3P-location 3 plant, L4P-location 4 plant, L1S-location 1 soil, L2S-location 2 soil, L3S-location 3 soil, L4S-location 4 soil.

As we can observe all soil samples are described by high content of iron, manganese, titanium and barium, while plant samples are defined by high content of potassium and calcium. The analyzed soil from location 2, has the highest content of rubidium, tantalum, lead and thorium compared to the other three analyzed soil samples, which recorded lower values.

The results show that L1 soil samples present high content of Cobalt, while L2 soil samples are characterized by high quantities of Fe, Cu, Mg. Vanadium was approximately twice as abundant in the soil sample from location 2 compared to other analyzed soils. Additionally, location 1 demonstrated the highest molybdenum content in nettle plants, and the mean strontium content was greater in plants from location 1 compared to others (Table 1).

The L3 soil samples elemental analysis result proves that Bazos (the protected area) is an unpolluted zone. The nettle plants collected from L3 present the highest content of potassium and calcium (Table 1).

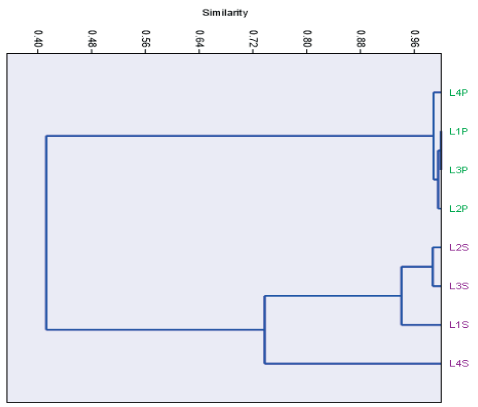

Figure 2. Graphical representation of cluster analyzes

Legend: L1P- location 1 plant, L2P-location 2 plant, L3P-location 3 plant, L4P-location 4 plant, L1S-location 1 soil, L2S-location 2 soil, L3S-location 3 soil, L4S-location 4 soil

Location 4 soil samples are described by the highest content of Ca, Mo, Sb showing a potential contamination with Sc.

The results of this study show that the nettle plants collected from the four different locations in Romania can be considered a source of valuable bio-elements. Nettle plants collected from different locations can be considered valuable sources of bio-elements due to their rich nutritional content, medicinal properties (Devkota et al, 2022), adaptability, sustainability (being used as an indicator of high soil fertility), and versatility in consumption (Schreiner, 1959 cited by FEIS, 2023).

The cluster analysis (Figure 2) was performed based on paired group algorithm and correlation as similarity measure. As we can observe the data group belonging to the nettle plants (LP 1-4) is more homogenous based on the mineral content compared to the cluster corresponding to the soil minerals data (LS 1-4).

Clustering soil and plant samples based on mineral concentrations provides a structured approach to understanding complex datasets in various scientific and agricultural contexts, like: *Pattern Recognition* (Butler et al., 2020), *Environmental Assessment* (Stein et al., 2017), *Precision Agriculture* (Gavioli et al., 2019), *Research and Hypothesis Testing* (Ahmad et al., 2022), *Verification of Food Safety* (Kuang et al., 2022) etc.

The fingerprint specific to each location is presented in Figures 3-6. The X-axis represents different minerals being analyzed in the mentioned samples, while y-axis represents the percentage composition of each respective mineral in the sample. The percentage indicates the proportion of each mineral relative to the total composition of minerals in the sample. The selected type of graph commonly used in elemental analysis, provides a visual represent-tation (fingerprint) of the relative abundance of different minerals, aiding in the interpretation of the sample's overall mineral composition.

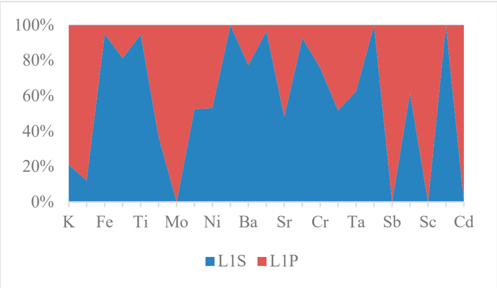

Figure 3. Mineral content fingerprint of the studied soil and plant samples collected from location 1 (Turcinesti - Gorj County)
Legend: L1P- location 1 plant, L1S-location 1 soil

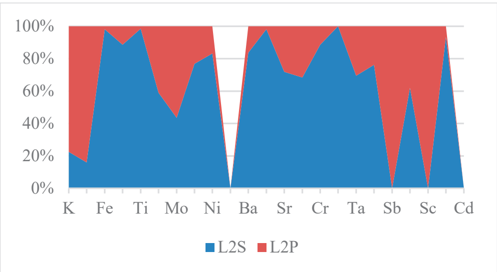

Figure 4. Mineral content fingerprint of the studied soil and plant samples collected from location 2 (Vadu-Dobrii - Hunedoara County)
Legend: L2P-location 2 plant, L2S-location 2 soil

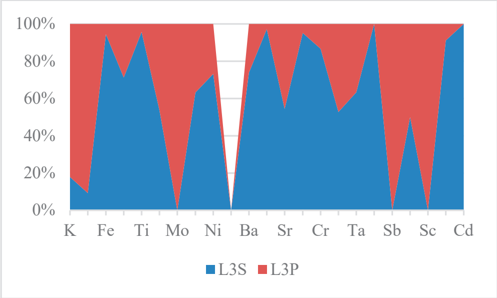

Figure 5. Mineral content fingerprint of the studied soil and plant samples collected from location 3 Bazos, Timis county
Legend: L3P-location 3 plant, L3S-location 3 soil

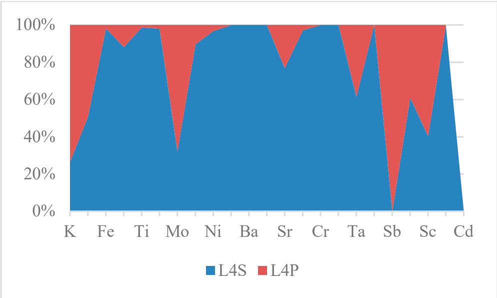

Figure 6. Mineral content fingerprint of the studied soil and plant samples collected from location 4 Timisoara, Timis County,
Legend: L4P-location 4 plant, L4S-location 4 soil

The General linear models (GLM) presented in Figures 7-10 describe the relation between soil and nettle plants for each samples collecting area from the perspective of multiple regression analysis, predicting the variation of "a dependent variable in terms of a linear combination (weighted sum) of several reference functions" (GLM-BrainVoyager v23.0).

For creating the GLM mathematical models, all variables and parameters were used. On the *x-axes* are distributed the data values corresponding to the to the minerals detected in soil samples and on *y-axis* are represented the data values corresponding to the minerals detected in plant samples.

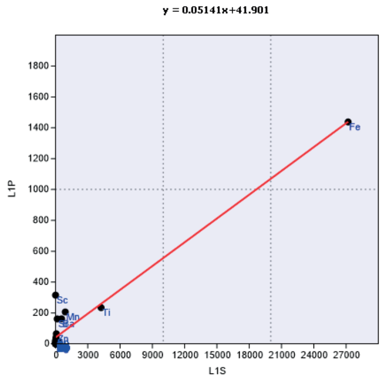

Figure 7. General linear model specific for location 1
Legend: L1P- location 1 plant, L1S-location 1 soil,
p (slope = 0): 7.548E-63

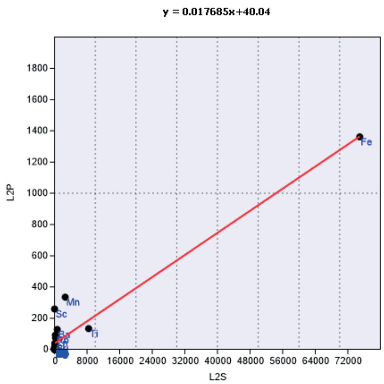

Figure 8. General linear model specific for location 2
Legend: L2P-location 2 plant, L2S-location 2 soil
p (slope = 0): 2.015E-52

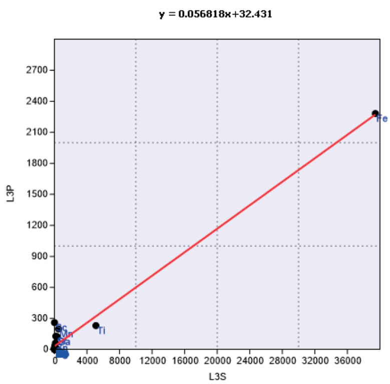

Figure 9. General linear model specific for location 3
Legend: L3P-location 3 plant, L3S-location 3 soil
p (slope = 0): 0

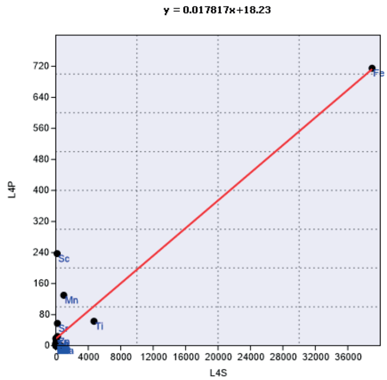

Figure 10. General linear model specific for location 4
Legend: L4P-location 4 plant, L4S-location 4 soil.
p (slope = 0): 0

The low p-value provides support for the presence of a statistically significant relationship between the predictor variable and the response variable in the presented GLM.

CONCLUSIONS

The X-ray fluorescence technique is rapid and offers multiple opportunities for elemental analysis, to identify the mineral uptake by plants or to detect the variations of pollution of various areas. As we can observe (figures 4-7), based on the mineral fingerprint of each location studied samples (soil and nettle plants) we can identify the plants desired to be used as food or medicine, or we can monitories the contamination level of soil and plants. Based on these observations we can suggest that the fingerprints created based on the XRF studies can generate maps of pollutants or desired minerals and to monitories the response of biocenosis to the environmental changes. The mathematical models based on spatial correlation are valuable tools to motorize both: cause and effect.

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PRELIMINARY RESULTS REGARDING THE EVOLUTION OF SMALL POTATO MERISTEMATIC EXPLANTS (0.1-0.2 MM)

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Abstract

This study has as its main objective the meristematic regeneration starting from small explants (0.1-0.2) to ensure viral eradication in potato culture. The experiment is monofactorial, the analyzed factor being the variety with 4 graduations: a1: Azaria; a2: Braşov; a3: Cosiana; a4: Cezarina (as control). For Azaria, Brasovia and Cosiana cultivars, the DAS-ELISA test was performed from potato tuber sprouts to determine the viral infection of these cultivars. Thus, the Cosiana variety presented the highest viral load, according DAS-Elisa test, 3 viruses specific to potato culture were identified: PVS, PVM and PLRV; this is followed by the Brasovia variety, which presented the PVS and PVM viruses, and for Azaria variety, the existence of PVS virus was observed. The inability to guarantee complete elimination of viral particles, especially in cases with mixed infections, remains a limitation for these methods based on meristem cultures. After each pass of the meristems, the statistical analysis was performed, regarding the regeneration of the meristems, finding after each pass the decrease in the viability of the meristems, the reason being the reduced sizes of the meristems.

Key words*: potato, virus, in vitro, meristem, regeneration.*

INTRODUCTION

Climate change and human population pressures are leading to rapid changes in agricultural practices and cropping systems that favour destructive outbreaks of viral diseases. Globalization of agriculture and international trade are spreading viruses and their vectors to new geographic regions, with unexpected consequences for food production and natural ecosystems (Jones and Naidu, 2019). Viruses account for nearly 50% of plant disease pathogens worldwide and damage natural vegetation as well as cultivated plants (Jones and Naidu, 2019).

Potato, being a vegetatively propagated crop, is highly affected by seed quality degeneration as more potato viruses accumulate in seed tubers with each generation, leading to reduced yield potential (Sakha et al., 2017).

Obtaining virus-free plants is necessary for successful viral disease management and sustainable propagation activities, including potato germplasm conservation and global exchange of genetic resources (Naik and Khurana, 2003; Volmer et al., 2017; Ellis et al., 2020).

The *in vitro* culture technique represents the most successful strategy for obtaining virus-free plants (Wang et al., 2018).

In methods based on meristem cultures, the size of the explant affects the efficacy of virus eradication. Excision of 0.2 mm shoot tips containing the apical dome with one or two leaf primordia is usually required (Wang et al., 2006; Zhang et al., 2019). For meristematic explant regeneration, the apical tip taken must contain at least 1-2 leaf primordia, which ensure the production of auxins and cytokinins (Bhojwani and Dantu, 2013). Excision of such small shoot tips is laborious, time-consuming and a highly skilled task. Also, results can be variable in terms of shoot growth and frequency of virus eradication (Bettoni et al., 2016; Magyar-Tábori et al., 2021).

The inability to guarantee complete elimination of viral particles, especially in mixed infections, remains a limitation for these meristem culture-based methods (Faccioli and Marani, 1998; Zhang et al., 2019).

All plants obtained by meristem cultures, with or without prior thermotherapy, must be tested to confirm the "virus-free" status. It is sufficient to confirm this status in the case of a single regenerant in order to obtain a virus-free clone further, through vegetative propagation (Badea and Săndulescu, 2001).

MATERIALS AND METHODS

To determine the capacity to regenerate small meristems, 4 potato varieties (created at National Institute of Research for Potato and Sugar Beet Brasov) were studied in a monofactorial experiment, with 4 gradations: a_1: Azaria; a_2: Braşov; a_3: Cosiana; a_4: Cezarina. As control, the Cezarina variety was established.

In the experiment carried out, for the statistical analysis of the obtained results, the variance analysis method (ANOVA monofact) was used. The statistical analysis was performed after each subculture.

According to the result of the DAS-ELISA test, the following viruses were identified: PVS virus for Azaria potato variety; PVM and PVS viruses for Brașovia variety; PLRV-PVA, PVM and PVS viruses in Cosiana potato variety.

The meristem sampling experience was carried out in the laboratory, under the conditions required for *in vitro* technology; the operations of sampling meristems and their inoculation were carried out in the sterile premises, previously prepared with the necessary materials.

The preparation of biological material for meristematic sampling includes several stages.

In the first stage, tubers were selected, belonging to the studied varieties. The tubers were allowed to sprout in the dark, at a temperature of 18^0C, until the sprouts reached 2-3 cm in length.

The next stage consisted in the preparation of the biological material for inoculation, which was achieved by sterilizing it. Disinfection of the biological material used was carried out by: immersing potato sprouts in a 0.1% sodium hypochlorite solution for 10 minutes; 3 successive washes in sterile distilled water; brushing spouts, on sterile filter paper. Aseptic dissection of the meristem is a delicate process and all operations were performed in the sterilized room, in the niche with laminar flow of sterile air, previously asepticized by irradiation with ultraviolet lamps, for 30 minutes. In the hood with laminar air flow, before the meristematic sampling procedure, it is preferable to start the air flow, 20-30 minutes.

Another stage was the excision of the meristematic explants and their inoculation in culture vessels (test tubes) with the aseptic medium. The excision of the meristems was carried out under a binocular magnifier (x10-40), in hood with sterile air flow and by using sterile equipment, on a surface that was previously disinfected with alcohol 70^0. The removal of the small leaflets surrounding the growth tip was carried out until the meristematic dome with leaf primordia was observed (Figure 1). The meristem must not exceed the size of 0.2, since the further we move away from the meristematic cells, the greater the danger of viral infection.

The initiation of the meristem culture was achieved by explanting the cauline meristems (apical or axillary) and inoculating them on a nutrient medium for plant regeneration (Figure 2).

The meristem represents an identical clone to the mother plant and can be preserved in a test tube, as the offspring of a healthy mother plant with satisfactory sanitary conditions. The advantage of micropropagation *in vitro* is that of the rapid multiplication, to infinity, of a material genetically identical to the plant from which it started, especially "rejuvenated", healthy and much more homogeneous material.

The culture of meristems therefore represents the starting point in obtaining a healthy material. The successful elimination of potato viruses depends both on the type of virus to be eradicated and on the size of the meristematic explant to be inoculated, which is the main factor that conditions the ability to obtain healthy plants.

For potato, the PVS, PVX and PVM viruses are eliminated by the culture of very small meristematic explants, 0.1-0.3 mm, and the chances of survival of these meristems and regeneration are low; PLRV and PVY viruses can be eradicated by sampling meristems larger than 0.5-3 mm.

The first subculture was performed one month after inoculation (Figure 3). As a regeneration agent, naphthyl acetic acid (NAA 0.5 mg/l) was applied to the culture medium. Each meristem receives a number, which when multiplied is called a clone.

From the inoculation stage to the formation of small meristematic buds, 10 subcultures were

carried out every 25 days. Explants inoculated on aseptic media, cultivated under environmental conditions favourable to their development, evolve over time.
Figure 4 shows the development of meristem four to five months after inoculation and formation of small buds (after 6-8 subculture). Compared to the first stage, initiation of the meristematic culture, in the second stage, namely the formation of buds, the culture medium plays a more important role. This stage consists in the maintenance and growth of stock inoculums. In the third stage, plantlets are formed, the buds elongate, becoming shoots; from this stage the *in vitro* multiplication begins. A few days after inoculation on fresh medium and after each subculture, test tubes with necrotic inoculums were selected, following the evolution and development of explants that survived. No infections of the inoculums were observed in test tubes.

Figure 1. Meristem (original photo)

Figure 2. Inoculated meristem (original photo)

Figure 3. Subculturing meristem on the fresh medium (original photo)

Figure 4. Bud formed from the meristematic explant (original photo)

RESULTS AND DISCUSSIONS

The statistical interpretation of meristem regeneration after the first subculture procedure (Table 1), highlights a significant positive difference for Brasovia variety (13.14%) compared to the control variety (Cezarina).
The influence of variety in the process of meristematic regeneration suggests, after the second subculture, the superiority of the Brasovia variety, which differs significantly positively (16.60%) compared to the Cezarina variety (Table 2).
The analysis of the influence of the variety on the survival of the meristems after the third subculture, places the Brasovia variety in first place, with a significant positive difference of 17.64%, compared to the control variety (Table 3).
At the 4th, 5th, 6th and 7th subcultures, it can be seen that values obtained regarding the regeneration capacity of the meristems are close, without significant differences (Tables 4, 5, 6 and 7). At the sixth subculture, Cezarina variety has the highest regeneration capacity (74.92%), followed by Azaria (67.46%), Brasovia (63.10%). In the seventh subculture, in the Brasovia variety, a decrease in viable meristems is observed; of those initially inoculated and passed, 75.89% necrosed.
At the sixth subculture, for Brasovia variety, the formation of plantlets from the meristematic bud was observed (Figure 5). Since each meristem receives a number, which is called a clone, a number that will be respected throughout the multiplication stage, the respective clone, the regenerated plantlet is multiplied by uninodal segmentation into mini cuttings (Figure 6), with the aim of forming and developing new plants.

The clones that generated plantlets are in percentage of 5.56% (relative to the number of meristematic samples) for Brasovia variety, and they were multiplied, going to generate new plantlets (Figure 7). Following the DAS-ELISA test carried out between 7-08.08.2023, the plantlets received the "virus-free" status, and they will be multiplied. Thus, the viruses with which the Brasovia variety was infected, respectively PVM and PVS, were eradicated for the tested clone. For the PVM and PVS viruses, the success of their elimination is difficult, because the meristems must be very small, and there is a risk that they will become necrotic.
At the 7th pass, for Azaria variety, a plantlet was formed from the meristem (in percentage of 4.34% compared to the total number of inoculated meristems for this variety. The plantlet developed from meristem was sectioned at the level of each internode, in mini cuttings, to obtain new plantlets, identical to the mother plant.
According to Table 8, in the 8th subculture, the lowest percentage of meristematic regeneration is observed at Brasovia variety (16.97%, and the difference of 83.03% shows that they have necrotized, the reason being the very size small dimension of meristems when sampling, to ensure the success of eradicating the virus, which is why in this variety, the plantlets developed from the meristem received the "virus-free" status).
In the 9th subculture (Table 9), the lowest percentage of meristematic regeneration (16.97%) is also observed at Brasovia variety. The Cezarina variety presented the highest meristematic regeneration capacity (27.65%), followed by the Cosiana variety (25.28%).
At the tenth pass, it is found that the values obtained regarding the meristem regeneration capacity are close, with no significant differences between the varieties (Table 10).
The losses incurred during the meristem subculturing procedure were due to tissue necrosis. Since meristematic sampling is performed under a microscope and the explant is very small, necrosis is rarely observed in the first subculture. Along the way, necrosis may also occur due to the stress for each explant, by transferring to fresh medium and possible wounding with the instruments, taking into account the small dimensions of the explant.

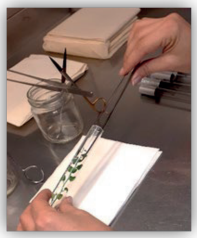

Figure 5. Plantlet developed from meristematic bud

Figure 6. Minicutting

Figure 7. Plantlets developed from mini-cuttings (original photo)

Table 1. Influence of genotype on meristem regeneration after the first subculture

Variety	Survival after the first subculture (%)	Diff.	Sign.	
Azaria	80.95	-5.91	ns	B
Brasovia	100.00	13.14	*	A
Cosiana	87.71	0.84	ns	B
Cezarina (Ct)	86.86	-	-	B

LSD (p 5%) = 9.92%; (p 1%) = 15.03%; LSD (p 0.1 %) =24.14%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 2. Genotype influence on meristem regeneration after 2nd subculture

Variety	Survival after 2nd subculture (%)	Diff.	Sign.	
Azaria	73.81	-9.59	ns	B
Brasovia	100.00	16.60	*	A
Cosiana	81.32	-2.08	ns	B
Cezarina (Ct)	83.40	-	-	B

LSD (p 5%) = 16.33%; (p 1%) = 27.73%; LSD (p 0.1 %) =39.73%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 3. Genotype influence on meristem regeneration after 3rd subculture

Variety	Survival after 3rd subculture (%)	Diff.	Sign.	
Azaria	73.81	-2.30	ns	B
Brasovia	93.75	17.64	*	A
Cosiana	74.38	-1.73	ns	B
Cezarina (Ct)	76.11	-	-	B

LSD (p 5%) = 15.21%; (p 1%) = 23.03%; LSD (p 0.1%) =37.00%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 4. Genotype influence on meristem regeneration after 4th subculture

Variety	Survival after 4th subculture (%)	Diff.	Sign.	
Azaria	71.03	-5.08	ns	A
Brasovia	79.17	3.06	ns	A
Cosiana	71.25	-4.86	ns	A
Cezarina (Ct)	76.11	-	-	A

LSD (p 5%) = 10.02 %; (p 1%) = 15.18%; LSD (p 0.1 %) =24.38%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 5. Genotype influence on meristem regeneration after 5th subculture

Variety	Survival after 5th subculture (%)	Diff.	Sign.	
Azaria	67.46	-7.46	Ns	A
Brasovia	63.10	-11.82	Ns	A
Cosiana	61.88	-13.04	Ns	A
Cezarina (Ct)	74.92	-	-	A

LSD (p 5%) = 28.11 %; (p 1%) = 42.57%; LSD (p 0.1 %) =68.39%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 6. Genotype influence on meristem regeneration after 6th subculture

Variety	Survival after 6th subculture (%)	Diff.	Sign.	
Azaria	67.46	-7.46	ns	A
Brasovia	63.10	-11.82	ns	A
Cosiana	59.10	-15.82	ns	A
Cezarina (Ct)	74.92	-	-	A

LSD (p 5%) = 29.85 %; (p 1%) = 42.50%; LSD (p 0.1 %) =72.61%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 7. Genotype influence on meristem regeneration after 7th subculture

Variety	Survival after 7th subculture (%)	Diff.	Sign.	
Azaria	50.40	1.53	ns	A
Brasovia	24.11	-24.76	ns	A
Cosiana	41.04	-7.82	ns	A
Cezarina (Ct)	48.86	-	-	A

LSD (p 5%) = 50.69 %; (p 1%) = 76.76%; LSD (p 0.1 %) =123.31%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 8. Genotype influence on meristem regeneration after 8th subculture

Variety	Survival after 8th subculture (%)	Diff.	Sign.	
Azaria	23.61	-15.69	ns	A
Brasovia	16.97	-22.33	ns	A
Cosiana	25.28	-14.02	ns	A
Cezarina (Ct)	39.30	-	-	A

LSD (p 5%) = 34.32%; (p 1%) = 51.97%; LSD (p 0.1 %) =83.49%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 9. Genotype influence on meristem regeneration after 9th subculture

Variety	Survival after 9th subculture (%)	Diff.	Sign.	
Azaria	23.61	-4.04	ns	A
Brasovia	16.97	-10.69	ns	A
Cosiana	25.28	-2.37	ns	A
Cezarina (Ct)	27.65	-	-	A

LSD (p 5%) = 37.18%; (p 1%) = 56.30%; LSD (p 0.1%) =90.44%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

Table 10. Genotype influence on meristem regeneration after 10th subculture

Variety	Survival after 10th subculture (%)	Diff.	Sign.	
Azaria	23.61	0.72	Ns	A
Brasovia	13.39	-9.50	Ns	A
Cosiana	25.28	2.39	Ns	A
Cezarina (Ct)	22.89	-	Ns	A

LSD (p 5%) = 41.40%; (p 1%) = 62.70%; LSD (p 0.1 %) =100.72%.
Means found in the same columns followed by the same letters are not significant according to the Duncan test ($p \leq 0.05$)

At the tenth subculture, meristems that survived for each variety developed small buds and a part of them form plantlets (16% for Azaria, 11.11% for Brasovia, 2.56% for Cosiana and 2.17 for Cezarina).

Figure 8 shows the behaviour of each variety regarding meristematic survival.

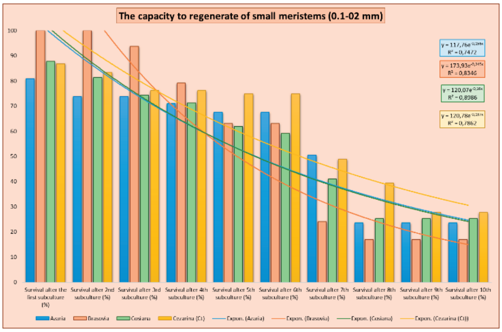

Figure 8. The survival of meristems

CONCLUSIONS

Up to the third subculture, the survival capacity of meristems was influenced by cultivar, but after the fourth subculture, there were no significant differences between cultivars.

Among the four varieties, the Brasovia variety presented the lowest survival capacity from the eighth to the tenth subculture (13.39%).

At the opposite pole is the Cosiana variety, which in the last subculture showed a superior survival capacity (25.28%).

The sampling of very small meristems (0.1-0.2 mm) leads to their loss, not being able to survive.

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ALLELOPATHIC EFFECT OF THE ESSENTIAL OIL OBTAINED FROM HYSSOP (*HYSSOPUS OFFICINALIS* L., FAM. *LAMIACEAE*)

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Abstract

The paper aimed to present the evaluation of the allelopathic effect of the essential oil (EO) of hyssop (Hyssopus officinalis L.) obtained from a new Romanian variety ('Catalina' variety), on seed germination and seedling growth. As biological material, seeds from two weed species were used: green foxtail (Setaria viridis), Johnson grass (Sorghum halepense) and two vegetable species: lettuce ('May King' variety) and spinach ('Matador' variety). The main compounds identified in EO were: cis-pinocamphone (34.57%), trans-pinocamphone (13.73%), along with ß-pinene (13.32%), β-phellandrene (9.51%) and germacrene-D (5.38%). The obtained results demonstrated that EO had an allelopathic effect, inhibiting/stimulating seed germination and subsequent seedling development. However, the concentrations that showed inhibition/stimulation effect were different depending on the seed species tested. The study demonstrated possible allelopathic effects of EO from hyssop 'Cătălina', on weed and vegetable seed germination. The obtained results can be used in the future, for the development of commercial products with bioherbicidal action, but additionally, thorough research is needed regarding the mechanism of action, EO encapsulation, testing in field conditions, etc.

***Key words**: EO, Hyssopus officinalis, 'Catalina' variety, chemical composition, germination and seedling growth.*

INTRODUCTION

Allelopathy refers to the inhibitory or stimulatory effect of one plant species on the germination, growth, and metabolism of another plant species, due to the release of allelochemicals into the surrounding environment (Maurya et al., 2022). Allelochemicals are substances extracted from plants, which have the ability to synthesize secondary metabolites such as phenolic acids, phenols, flavones, flavonoids, saponins, coumarins, which accumulate in small quantities in the cells of various organs (roots, leaves, flowers, and seeds). These can release allelochemical compounds into the surrounding environment through leaching, root exudation, volatilization, decomposition of residues, or organic matter, in both natural and agricultural systems (Sakihama et al., 2002). Allelopathy is a complex process, and to date, a number of allelochemicals have been identified, which have been the subject of many studies, including species from the fam. Poaceae (Hierro et al., 2021; Fatholahi et al. 2020; Favaretto et al., 2018). However, very little data is known about the transport and biodegradation of allelochemicals in the soil or about the genetic populations of species with allelopathic properties (Acheuk et al., 2022).

The ability of allelochemical compounds to affect germination, inhibit, or delay the growth of other plants can be a problem for agricultural production due to weed interference with crop plants. However, these plant metabolites can also act as natural bioherbicides. Establishing practical ways to use allelochemicals in the field, the rapid adaptation of weeds to avoid them, the diversity of soil microbial communities, all these aspects should be researched in the future. The research conducted so far has shown that allelopathy has good potential for application in agricultural practice. Many crops with allelopathic potential have been used in agricultural production, but their application is limited to small-scale, regional areas (Kesraoui et al., 2022). Weed management involves using multiple techniques to limit the infestation of agricultural crops and minimize competition. These techniques have evolved to mitigate production losses and intervene only when a problem is identified (Abd-ElGawad et al., 2021). Currently, researchers are addressing weed control through physical, chemical, and biological methods. Weed control carried out in an environmentally friendly manner is considered a challenge. Natural resources provide new approaches for producing environmentally friendly and safe bioherbicides that are effective against weeds. One of these resources is represented by medicinal plants that produce essential oils (EOs), complex mixtures of secondary metabolites containing mono-, di-, and sesqui-terpenes, in addition to hydrocarbons (Assaeed et al., 2020; Abd-ElGawad et al., 2019). EOs and their volatile compounds are at the forefront due to their phytotoxicity and allelopathy, combined with rapid degradation in the environment, which can have practical applications in agriculture, horticulture, and ecology (Stan (Tudora) et al., 2022). They can be used as natural alternatives to synthetic herbicides and pesticides or as additives to improve crop quality and yield (Soares et al., 2023). The allelopathic effect of hyssop EO refers to its ability to influence the growth and development of other plants by releasing chemical substances into the surrounding environment. Thus, its antiviral, antiseptic, anti-inflammatory, and antispasmodic properties can be beneficial for human health as well as for weed control or crop stimulation. According to a study, hyssop EO exhibited inhibitory effects on the germination and growth of wheat, barley, oats seedlings, as well as on their photosynthesis and respiration. This effect was attributed to the high content of thymol and carvacrol, which acted as allelopathic agents. The study suggests that hyssop EO can be used as a natural herbicide in combating monocotyledonous weeds (Zheljazkov et al., 2021). Another study showed that the same type of EO had a stimulatory effect on the germination and growth of seedlings of dill, fennel and basil. The study suggests that hyssop EO can be used as a natural additive to improve the quality and yield of dicotyledonous crops (Raut & Karuppayil, 2014). In the study conducted by Almeida et al., in 2010, the main monoterpenic compounds identified in hyssop EO were β-pinene, *iso*- and *trans*-pinocamphone, compounds with phytotoxic activity on *Raphanus sativus, Lactuca sativa, Lepidium sativum*.

The use of EOs obtained from MAPs (Medicinal and Aromatic Plants) can be an important component in integrated weed management systems, and the identified bioactive compounds could be used as potential bioherbicides (Maurya et al., 2022). Currently, more than 1400 biopesticides have been registered worldwide (Balog et al., 2017), with a smaller number registered in Europe due to the complex regulations within the EU.

Thus, in the EU, there are approximately 60 biopesticides available, compared to over 200 biopesticides on the North American market (Kumar et al., 2021). At the beginning of this decade, the global biopesticide market exceeded 4 billion USD, with expected doubling by the year 2025 (Rakshit et al., 2021).

In this context, the aim of this study was to evaluate the EO obtained from the first Romanian variety of hyssop ('Cătălina' variety), aiming to assess: the extraction yield, compound analysis using GC/MS (chemical composition, identification of major compounds and compound classes), and its allelopathic capacity (% germination, radicle and plumule length).

MATERIALS AND METHODS

The hyssop EO, 'Cătălina' variety, was obtained through hydrodistillation. The plant material required for hydrodistillation was cultivated on the lands within INMA Bucharest (Băneasa area), on a reddish-brown forest soil, under the climatic conditions of the year 2021. The crop was established using seedlings produced by SCDL Buzău, following all technological steps. The aerial parts of the plants (inflorescences and shoot tips) were processed when harvested in the flowering stage. The extraction yield was calculated using the formula:

$$\text{Extraction yield} = V / M \text{ (ml/kg)}$$

where:

V = the volume of essential oil obtained from the sample of green plant (ml);

M = the mass of the sample of medicinal plants (kg).

The chemical composition of hyssop EO. The concentration of compounds was determined by GC/MS analysis. The analysis conditions were as follows: Gas chromatograph Agilent Technologies type 7890 A GC system, MS Agilent Technologies type 5975 C Mass Selective Detector; HP 5MS column 30 m x 0.25 mm x 0.25 µm (5% Phenylmethylsiloxane); injector temperature 250°C, detector temperature 280°C; Temperature regime: 25°C (10 degrees/min) to 280°C (const. 5.5 min); mobile phase - helium 1 ml/min; injected volume - 0.1 µl EO; split ratio - 1:100.

The biological material used in the experiments consisted of seeds from two weed species:
- Green foxtail (*Setaria viridis* L., fam. *Poaceae)*, an annual grass weed with drought resistance, heat-loving characteristics, forming sparse tufts. It prefers calcium-poor, light soils, rich in nutrients, in warm regions;
- Johnson grass (*Sorghum halepense* L., fam. *Poaceae)*, an invasive perennial grass weed that forms rhizomes and prefers warm areas. It reproduces through seeds and rhizomes, making it difficult to control,

and two vegetable species:
- Lettuce (*Lactuca sativa* L., fam. *Asteraceae*, 'May King' variety), an early variety with medium-sized heads, light green in color, intended for outdoor cultivation. The crop can be established both in spring and autumn. Sowing period: March, August, September. Planting distances: 40 cm/25-30 cm.
- Spinach (*Spinacia oleracea* L., fam. *Amaranthaceae,* 'Matador' variety), a semi-early variety with a vegetation period of 45 days. The rosette is medium-sized, with slightly wrinkled, oval, fleshy leaves, short-petioled, dark green in color. It is resistant to low temperatures, can overwinter in the field, and produces floral stems late. It is sown in the field in February-March or August-October. Planting distances: 15-20 cm/3-5 cm. It is used for fresh consumption as well as for processing.

The experimental protocol aimed to: sterilize the seeds by immersing them for 3 minutes in a solution of NaOCl, followed by rinsing with sterile distilled water and drying on sterile filter paper. Hyssop EO was homogenized with Tween 80 (1:1 V/V) and tested at four concentrations: 100, 150, 250, and 300 µl, using sterile distilled water, while Tween 80 in water was used as a control. In Petri dishes, 20 seeds and 5 ml of each concentration of solution were placed on sterilized filter paper, followed by sealing with Parafilm and incubation in a plant growth chamber (climatic chamber with Fitotron system, model KK 115), at 27ºC, with a humidity range of 70-80%. After 5 days, the germinated seeds (radicle > 2mm) were counted, and after another 12 days (at the end of the experiment), the lengths of the radicle and plumule were measured. Each concentration was tested in 3 replicates. The parameters monitored were germination (%), radicle length and plumule length (cm).

The formula was applied to calculate germination, expressed in percentage (Siddiqi et al., 2007):

Germination = (number of germinated seeds/total number of seeds)× 100, (%)

The statistical processing for the interpolating the experimental data regarding the influence of hyssop EO action on the seeds, was performed using the MathCAD Soft 2000 and Excel programs from the MS Office 2007 package (MathCad, 2000).

RESULTS AND DISCUSSIONS

Extraction yield of hyssop EO, 'Cătălina' variety

Following the hydrodistillation process of the fresh plant material, represented by inflorescences and shoot tips, the hydrolate (a mixture of essential oil and floral water) was obtained. The hyssop EO, thus obtained, was decanted, filtered, and then stored in dark-colored bottles and kept in a cool, dry place. The extraction yield obtained was 10 ml of EO per 1 kg of fresh plant material and 2.0 liters of floral water per 1 kg of plant material, for 'Cătălina' variety. The quantity and especially the quality of the essential oil and floral water obtained from the new variety of hyssop were influenced by the type of soil, agricultural techniques applied, as well as by the agro-climatic conditions present in the production year (2021).

Chemical profile of hyssop EO, ′Cătălina′ variety

Specialized literature (Stan (Tudora) et.al, 2022) mentions compounds characteristic for hyssop EO to include *iso*- and *trans*-pinocamphone, pinocamphone, and their precursor, β-pinene (Figure 1). Among other constituents are pinocarvone, sabinene, germacrene-D, germacrene D-4-ol, α- and β-phellandrene, thymol, carvacrol, elemol, limonene, linalool, and 1,8-cineole (Fathiazad & Hamedeyazdan, 2011; Ogunwande et al., 2011*)*.

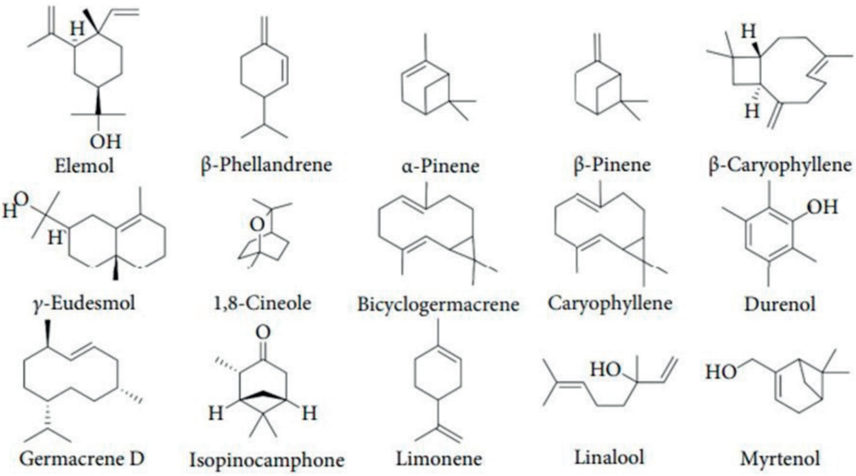

Figure 1. Chemical structures of the main bioactive compounds in hyssop EO (Source: Sharifi-Rad et al., 2022)

The chemical composition of hyssop EO is globally monitored by ISO 9841:2013.

In this document, 13 compounds are recognized as standards, and pinocamphone, *iso*-pinocamphone, and β-pinene are considered to be the most abundant compounds (40-90%).

For the EO obtained from the plants of the new variety of hyssop (produced in 2021), 16 compounds were identified, representing 99.99% of the total separated compounds.

The major compounds were: *cis*-pinocamphone (34.67%), *trans*-pinocamphone (13.73%), β-pinene (13.32%), germacrene-D (5.38%).

In addition to these, other compounds were also identified, but in variable amounts: β-phellandrene (9.51%), β-elemene (5.24%), α-phellandrene (2.64%), myrcene (2.72%), pinenol (1.37%), β-caryophyllene (1.92%), aromadendrene (1.70%), thujenol (1.28%), elemol (1.17%) (Table 1).

The main classes of compounds were: monoterpenes (84.58%), especially oxygenated ones (53.92%), and sesquiterpenes (15.41%), especially sesquiterpenes hydrocarbonate (16.24%) (Table 1).

Table 1. Chemical composition of the EO isolated from aerial part of the hyssop (*Hyssopus officinalis* L., fam. *Lamiaceae*), 'Cătălina' variety

Compound name[a]	**EO Hyssop ′Cătălina' variety**	
	RT	**Area %**
β-Pinene	*9.59*	*13.32*
α-Phellandrene	10.66	2.64
Myrcene	13.45	2.72
D-Limonene	14.75	1.43
β-Phellandrene	*15.10*	*9.51*
β-Ocimene	17.23	1.04
Carenol	21.36	2.87
trans-Pinocamphone	25.16	13.73
cis-Pinocamphone	25.94	34.67
Pinenol	26.49	1.37
β-Caryophyllene	27.44	1.92
Aromadendrene	28.59	1.70
Germacrene-D	*30.09*	*5.38*
β-Elemene	30.64	5.24
Thujeol	31.75	1.28
Elemol	37.73	1.17
Total major compounds	**16 compounds identified 99.99%**	
Monoterpenes hydrocarbons	30.66	
Oxygenated monoterpenes	53.92	
Monoterpene	**84.58**	
Sesquiterpenes hydrocarbons	14.24	
Oxygenated sesquiterpenes	1.17	
Sesquiterpenes	**15.41**	

[a]Compounds identified based on retention index.

In the study conducted by Khan et al., in 2012, for the EO obtained from hyssop from Kashmir, the main compounds were: myrcene (2.24%), terpinene (1.03%), (Z)-sabinene hydrate (2.02%), linalool (1.04%), terpinen-4-ol (2.55%), germacrene-D (1.61%), and elemol (3.43%). The study also showed that the extraction yield was affected by the harvest stage, as young plants in the pre-flowering stage had a lower yield in obtaining EO compared to older plants, in full bloom and post-bloom. It was also observed that pinocamphone (the main compound in hyssop oil) gradually increases until after the post-flowering stage, while β-pinene, quantitatively in the plant, first increases and then decreases after the stage of full flowering. Hyssop EO from Bulgaria has as main constituents: 1,8-cineole (39.6%-48.2%), *iso*-pinocamphone (16.3%-28.0%), and pinene (9.4%-11.4%). A similar composition was found in a population of hyssop growing in eastern Serbia, with 1,8-cineole (36.4%), pinene (19.6%), and *iso*-pinocamphone (15.3%) being the most important compounds (Dzamic et al., 2013). Another study conducted on EO from Bulgaria, carried out by Hristova et al. in 2015, found 29.1% *iso*-pinocamphone, 11.2% *trans*-pinocamphone, and 18.2% β-pinene, while the concentration of these compounds in the EO from our study was 34.67%, 13.73%, and 13.32%, respectively (Table 1). The results of the study conducted by Venditti et al., in 2015 on two subspecies of hyssop from Italy (subsp. *officinalis* and subsp. *aristatus*) showed the presence of monoterpenic ketones such as pinocamphone, *iso*-pinocamphone, and methyl eugenol in the EOs. In another study conducted in Italy on EOs of hyssop cultivated at two different altitudes (100 m and 1000 m), the percentage variation of the main compounds was observed: 34-18.5% pinocamphone, 3.2-29.0% *iso*-pinocamphone, 10.5-10.8% β-pinene, 7.4-9.6% α-phellandrene, thus demonstrating the significant effect that the environment has on the composition of EOs (Sas-Piotrowska et al., 2010). In the study conducted on EO obtained from the aerial parts of hyssop plants cultivated in Egypt (Hussein et al., 2015), 33 compounds were identified, representing 99.99% of the total EO. The major compounds were *cis*-pinocamphone (26.85%), β-pinene (20.43%), and *trans*-pinocamphone (15.97%). The study conducted in Poland by Zawiślak in 2013 showed that the main compounds were: *cis*-pinocamphone (33.52%-37.13%), *trans*-pinocamphone (23.43%-28.67%), β-pinene (7.89%-8.12%), elemol (5.86%-8.95%), germacrene-D (3.23%-4.65%), and caryophyllene (2.67%). The Romanian EO of *H. officinalis* mainly contains aliphatic fatty acids: eicosadienoic acid (0.68%), linolenic acid (63.98%), arachidic acid (2.64%), stearic acid (10.73%), and palmitic acid (15.60%) (Benedec et al., 2002). In the study conducted by Jop et al. in 2021, the compounds *cis*-pinocamphone, *trans*-pinocamphone, and β-pinene were confirmed as the main three active, dominant compounds in the EO of *H. officinalis*, which is in accordance with the results of other researches (Hajian-Maleki et al., 2019; Kizil et al., 2010; Mahboubi et al., 2011;), but in different proportions. Monoterpenes (pinocamphone, *iso*-pinocamphone, etc.), the main representatives of this type of EO, are relatively rarely detected in larger quantities in EOs of other species. In terms of numbers (the most), 44 compounds have been identified in hyssop EO. In addition to the compounds already mentioned, other monoterpenes (β-pinene, pinocarvone, myrcene, etc.) have also been encountered, but in small concentrations. Thus, the biological variability of hyssop EO is relatively low, as indicated by available data (Hüsnü & Buchbauer, 2020). Although there are numerous studies regarding the successful use of EOs in weed control, there are constraints that limit their practical application. The role of EO composition is not clearly defined, and the mechanisms of action and selectivity are poorly understood, thus limiting their implementation. Additionally, studies regarding the potential side effects of these EOs on beneficial soil microorganisms are still lacking (De Mastro et al., 2021). In conclusion, all the data presented above support and confirm the results obtained in the present study regarding the chemical composition of the EO obtained from the first Romanian variety of hyssop, 'Cătălina' variety.

The allelopathic activity of EO of hyssop (Hyssopus officinalis L.), 'Cătălina' variety

According to Abd-ElGawad et al. (2021), hyssop essential oil containing monoterpenes such as β-pinene, *iso*-pinocamphone, and *trans*-

pinocamphone as main compounds, exhibits significantly higher phytotoxic activity on certain target plant species (*Raphanus sativus, Lactuca sativa, Lepidium sativum*). The monoterpenes (carvacrol, terpinene, thymol, etc.) are known to disrupt the cell membrane and affect photosynthesis, cellular respiration, and mitosis (De Mastro et al., 2021; Werrie et al., 2020). However, not much is known about the mechanism of action on plant metabolism (Lins et al., 2019), but it seems that they have cytotoxic effects that harm several plants (Acheuk et al., 2022; Sampaio et al., 2021). Similar to other monoterpenes, carvacrol has strong inhibitory effects on germination and seedling growth (Atak et al., 2016). Linalool is a monoterpene that affects multiple metabolic pathways, reducing photosynthesis, respiratory activity, and altering water status (Singh et al., 2002).

The effect of hyssop EO on seed germination

The results regarding the effect of treatment with EO obtained from hyssop on the germination of different seed species are presented in Figure 2.

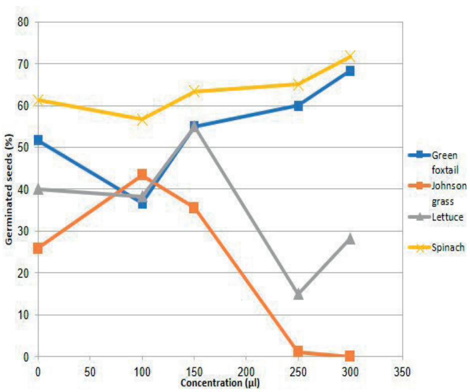

Figure 2. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on seed germination

For Green foxtail, it is observed that up to the concentration c=100µl, the effect is inhibitory, leading to a reduction in germinated seeds (38%). Then, as the concentrations increase, the effect switches to stimulation of germination, reaching 70% germinated seeds at c=300 µl.

For Johnson grass, the situation is the opposite. At c=100 µl, germination is stimulated (42%), then it slightly decreases to 35% at c=150 µl. At c>150µl, there is a significant reduction in germination, with almost complete inhibition observed. Thus, at c=300 µl, only 2-3% of seeds are germinated.

For lettuce, there is an interval of concentration between 100-150 µl where the percentage of germinated seeds increases to 55%, indicating stimulation of germination. This is followed by a sudden decrease in germination (below 15%) at c=150-250 µl, indicating inhibition.

For spinach, at c>100 µL, there is an increase in the percentage of germinated seeds with the concentration used in the treatment (stimulation effect), reaching 72% for c=300 µl.

In the study conducted by Zheljazkov et al., 2021, it was demonstrated that *H. officinalis* EO applied at a dose of 90 µl completely suppressed the radicle growth of barley and wheat and germination (%), compared to the control. It is interesting to note that the same EO applied at a dose of 10 µl did not reduce germination in wheat, but it did reduce the number and length of radicles, seedlings, and vigor index. In another study conducted by Argyropoulos et al., 2008, the effect of mint EO (*M. spicata*) on the weed species *Setaria verticillata* was evaluated, and the results showed that the EO (with 82% *trans*-piperitone oxide) severely inhibited all tested species.

The effect of hyssop EO on radicle length

The experimental results aimed at assessing the effect of hyssop EO on radicle length (cm) are presented in Figure 3.

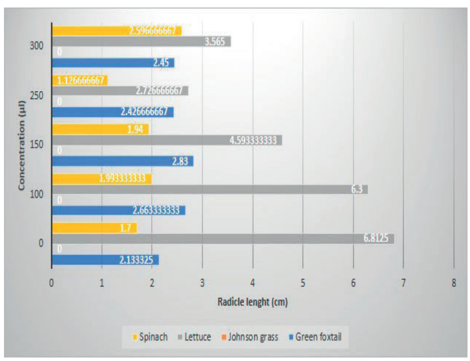

Figure 3. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on radicle length

For Green foxtail, the treatment at c=100-150 µl had a stimulating effect, with an increase in radicle length of 2.83 cm. As the concentration of EO in the tested solutions increased, the effect was reversed, leading to a reduction in radicle growth. Thus, at c=350 µl, the radicle growth was only 2.45 cm. Similar results were obtained by Koiou et al., 2020, with tests conducted on *Setaria verticillata*, showing that germination and root length were mostly reduced as the concentrations of compounds in EOs increased, but not proportionally.

For Johnson grass, the result is consistent with the previous one, meaning that at c>100 µl, complete inhibition of radicle growth was observed. For lettuce, the effect is inhibitory, and it is noticeable that as the concentration of EO increases, the radicle length decreases, so that at c=250 µl, the radicle is only 2.73 cm compared to the control (6.81 cm). For spinach, at c=100-150 µl (radicle length is 1.99 cm), the effect is stimulatory, then decreases to 1.12 cm (c=250 µl, and finally increases to 2.60 cm (c=300 µl).

Specialized literature mentions few studies related to the action of *H. officinalis* EO on the germination and radicle growth of some garden vegetable species (*Raphanus sativus, Lactuca sativa,* and *Lepidium sativum*) (Zheljazkov et al., 2021; Hristova et al., 2015).

In the tests conducted with hyssop EO, it did not inhibit the germination of *Lactuca sativa* L. and *Lolium perenne* L. seeds, although it slightly decreased (≈20%) root and leaf growth in *L. perenne* (De Elguea-Culebras et al., 2017). Amri et al., in 2013, demonstrated the phytotoxic capacity of various EOs and attributed to the compound 1,8-cineole (also present in hyssop EO) a strong allelopathic effect. Additionally, the increased content of pinocarvone in hyssop EO could also be linked to certain allelopathic effects observed previously for various carvone isomers (Vokou et al., 2003).

The effect of hyssop EO on plumule length

The results obtained from the treatment with hyssop EO on plumule length in two weed species and two vegetable species are presented in Figure 4.

De Elguea-Culebras et al. in 2017 reported that 1,8-cineole (53%) and β-pinene (16%) are the major bioactive compounds present in hyssop EO, which have insecticidal effects on *Spodoptera littoralis* (cotton leafworm). Additionally, they reported that this type of EO does not have an inhibitory effect on the germination of *Lactuca sativa* L. var. *carrascoy* and *Lolium perenne* L. seeds, but it does have a slight inhibitory effect on root and leaf growth in this weed.

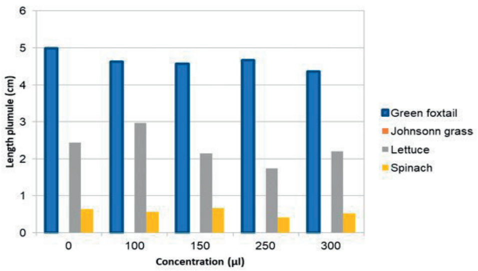

Figure 4. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on plumule length

As shown in Figure 4, for green foxtail, the treatment effect was insignificant, with results similar to the control. However, a slight inhibitory effect on plumule length (up to 0.5-0.6 cm maximum) is noticeable at all tested concentrations.

For Johnson grass, the results correlate with those obtained previously. Even though the germination process was initiated and even stimulated at c=100 µl, subsequent development of radicles and plumules was not allowed, resulting in a total inhibition of further seedling growth.

For lettuce, at c=100 µl, the response was the most favorable, as it shows a stimulation of 0.5 cm in plumule length. However, as the concentration increases, the length of the plumule decreases.

The experimental results obtained for spinach show no variation, meaning that the effect is not significant compared to the control.

The studies conducted have shown that monoterpenes, which are the major compounds in EOs, inhibit seed germination and subsequent seedling growth. These are secondary metabolites commonly described for their bioherbicidal activity (De Mastro et al., 2021; Kumar et al., 2020). The experimental results obtained from tests conducted with the aqueous extract obtained from the aerial parts of hyssop, on the species *Cucumis sativus* L. and *Triticum aestivum*, showed that it had an inhibitory effect on germination and root elongation (*T. aestivum* > *C. sativus*) (Islam et al., 2022; Dragoeva et al., 2010). In the study conducted by Tkachova et al. (2022), the influence of the allelopathic activity of aqueous extracts (leaves, stems, flowers) of hyssop on the soil, in the rhizosphere zone, was demonstrated, affecting the root growth of *Lepidium sativum* L. Typically, the accumulation of hyssop EO occurs in the flowers and leaves, and to a lesser extent in the stems. The accumulation of substances with inhibitory effects happens gradually, with the plant being capable of accumulating essential oil starting from the second year of cultivation. On average, the allelopathic activity index of water-soluble biologically active substances from the above-ground organs of hyssop plants in the first and second years of growth had a stimulatory effect at a concentration of 1:50 and an inhibitory effect when using plants from the third year of growth at a concentration of 1:10. This result confirms that the highest amount of allelochemical substances, which can negatively affect the growth and development of other plants, accumulates starting from the third year of cultivation. It should be noted that despite the difference in allelopathic activity of extracts obtained from different organs of the hyssop plants, the trend of its increase occurs with each year of cultivation in monoculture.

In conclusion, the most likely cause of the allelopathic activity of the new variety of hyssop ('Cătălina' variety), whether it is inhibition or stimulation of seed germination and subsequent seedling growth, is attributed to the action of the main compounds (*cis*- and *trans*-pinocamphone, β-pinene, germacrene-D), as well as to the synergistic action among them and the secondary compounds identified in smaller quantities in this EO (α and β-phellandrene, β-elemene, myrcene, β-caryophyllene, aromadendrene, pinenol, thujenol, elemol).

CONCLUSIONS

The results of this study have shown that the EO obtained from the new variety of hyssop ('Cătălina' variety) contains the main compounds: *cis*- and *trans*-pinocamphone, β-pinene, germacrene-D, along with other secondary compounds found in smaller quantities (α and β-phellandrene, β-elemene, myrcene, β-caryophyllene, aromadendrene, pinenol, thujenol, elemol).

Furthermore, the allelopathic effect of this EO is either inhibitory or stimulatory on seed germination and subsequent seedling growth, with the effect being influenced by the concentration used and the species to which the treatment is applied.

Further research should focus on harnessing the cultivation of the new variety of hyssop, developing stable, environmentally friendly, and effective bioherbicides that can be used in field conditions to control a wide range of target weeds. The use of allelopathy in agriculture can be achieved by incorporating allelopathic plant residues into the soil or through mulching, applying EOs obtained from plants with allelopathic effects, and using artificially produced allelochemicals (through organic synthesis, fermentation, tissue cultures, etc.). Importance should also be given to promoting the production of bioherbicides and raising awareness among farmers about their ecological benefits.

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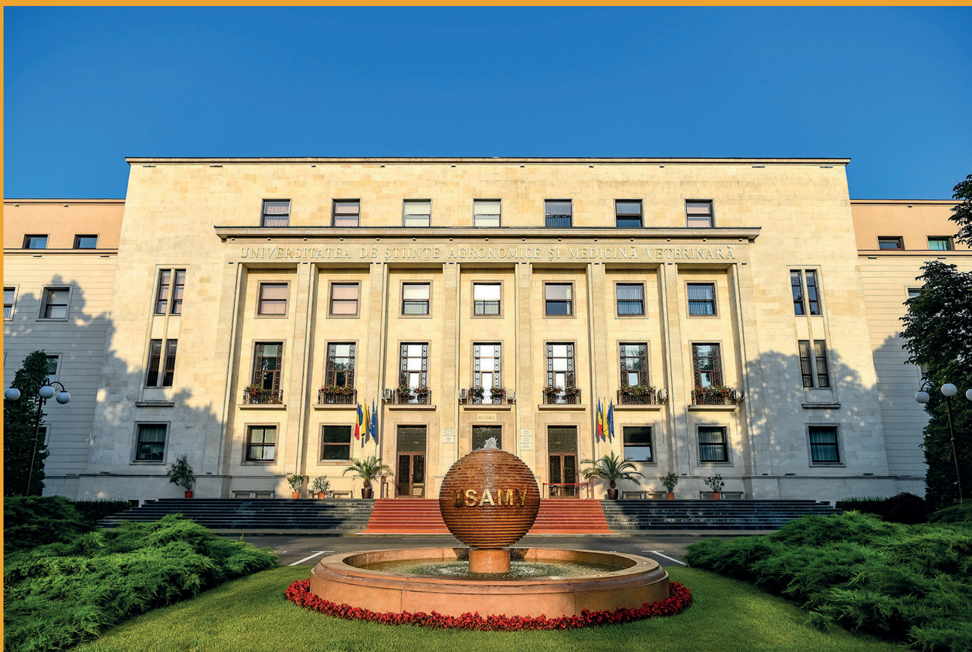

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